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Effects of High Pressure Processing on the Quality of Farm-Raised Abalone (Haliotis rufescens)

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EFFECTS OF HIGH PRESSURE PROCESSING ON THE QUALITY OF FARM-RAISED ABALONE (*Haliotis rufescens*)

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An Abstract of the Dissertation Presented
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High pressure processing (HPP) is a non-thermal method used to increase food safety and shelf-life. HPP has been explored for meat tenderization, with conflicting results depending on rigor status during HPP. In the seafood industry, HPP is used to shuck oysters and lobsters, but has been minimally investigated for use in processing abalones. Abalones are shellfish with high perishability that are typically sold live or frozen in the U.S. HPP has been proposed as an alternative method to process shucked abalone meats. The objectives of this research were to evaluate the effects of 1) rigor status during HPP on abalone quality, 2) of HPP on subsequently cooked abalone, 3) processing pressures of <500 MPa on shelf-life, and 4) HPP followed by papain treatment on physicochemical qualities of abalone.

In study 1, abalones were processed either pre- or post-rigor at 100 or 300 MPa for 1-5 min. Processing abalones pre-rigor caused significant ($p<0.05$) toughening of the foot compared to the control and post-rigor treatments. Foot color lightened significantly

as pressure increased, and was lighter in the post-rigor treatments. In study 2, uniform pieces of HPP abalone were boiled for 15 s. HPP did not affect cooked meat texture, but did cause significant increases in lightness of cooked treatments. For study 3, shelf-life of HPP abalone was evaluated over 35 days at 2ºC using microbiological, biochemical, and physical quality parameters. HPP abalone meat processed at 300 MPa did not exceed freshness indices for 25 days. In the final study, HPP abalones were vacuum tumbled with papain solutions (0.5% or 1.0% w/v), resulting in significantly more tender meat than in the controls. The proteolytic effect of papain was qualified by protein gel electrophoresis and scanning electron microscopy.

The results of these studies have important implications for the abalone industry. HPP could be used to improve the shelf-life of shucked abalone, as well as to lighten color without chemical bleach. Subsequently cooked HPP abalone meat did not differ in texture compared to cooked non-HPP meat, and papain treatment at the concentration and activity used in the final study effectively tenderized HPP abalone meat.

DEDICATION

I dedicate this manuscript with sincerest gratitude to my husband, Chris Mishoe.

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CHAPTER 1

INTRODUCTION

Shellfish are an expensive source of protein with high perishability. Processing is one way to overcome the perishability of shellfish and increase distribution. Common processes include canning, drying, and freezing which are economical but can negatively affect shellfish quality. Canning and drying are thermal processes which kill bacteria and denature protein with heat. Freezing is a preservation process used frequently by the seafood industry but it must be done quickly using cryogens, plates, or blast freezing for optimal quality. A newer non-thermal processing technology that has demonstrated promise in seafood processing is high pressure processing (HPP). HPP has demonstrated efficacy in processing oysters and lobsters but has not been universally adopted by the shellfish industry. Shellfish make excellent candidates for non-thermal processing since these technologies are currently expensive and are typically reserved for high value products. The advantages of HPP are many, but pasteurization and shell removal (shucking) are the most relevant to the shellfish industry. HPP is used regularly to pasteurize and shuck oysters, and in Maine is used to shuck lobsters.

1.1 Abalones

Abalones are molluscan shellfish related to sea snails. They belong to the genus *Haliotis*, and while there are nearly 100 known species of abalones, *H. rufescens* (red abalone) is the most commercially important in the western hemisphere (Tegner and others 1992). Abalones are a high value delicacy and one of the most expensive shellfish in the world. In the United States, live cultured abalones are sold for $25-40/lb, while

tenderized abalone steaks routinely sell for $100-125/lb (Catalina Offshore Markets 2010, Giovanni's Fish Market and Gallery 2012). Recent estimation of total worldwide production of abalones is 44,510 metric tons, of which 30,760 metric tons are cultured (Cook and Gordon 2010). Of the total abalone farmed worldwide, China produces approximately 23,000 metric tons per year with Australia, Chile, South Africa, and the United States (California) generating the remaining ~7,000 metric tons between them (Cook and Gordon 2010). The tremendous growth in cultured abalone in China is affecting world market prices of abalones, and Cook and Gordon (2010) recommend that abalone farmers diversify their product offerings to compete with the influx of available abalone meat on the market.

Typically, abalones are sold live in-shell, dried, canned, or frozen. Live abalones are desirable for chefs and consumers who wish to shuck and prepare their shellfish on-site, whether serving it raw as sashimi or tenderized and cooked. Some restaurants or wholesalers may have tanks to keep the abalones alive until they are needed, or they may order only what they need at a time. The freshness of the meat is superior to processed meat, however there are many downsides to purchasing live animals. Live abalones, particularly those imported to the United States from Asia, are expensive to ship. In preparation for shipment, the abalones are not fed for several days, decreasing glycogen concentrations in the muscle, and they may or may not be anaesthetized to minimize stress during the packing process (Brown and others 2008). The packaging material may be moistened to prevent desiccation of the gills, and oxygen may be used to reduce stress. Abalones have evolved to last long durations of time without water, but if they sustain

physical damage during handling, mortality rates may rise during shipment as they dehydrate (Brown and others 2008).

Once the abalones are unloaded, they may be shucked immediately and prepared for consumption, or placed in tanks to revive until a later time. The abalones must be shucked and cleaned, and it is estimated that the finished trimmed steak is only 15% of the original live weight of the abalone (Seafood Network Information Center 2000). Alternatively, abalones may be harvested, shucked, and further processed at the farm. Different unit processes result in distinctly different textural properties of the meat. Raw abalone meat is described as firm and crisp, while mechanically or thermally tenderized abalone meat is described as soft and chewy (Brown and others 2008). These textural attributes are quintessential to the acceptance of abalone meat by consumers. Abalone meat is naturally tough due to its high collagen content, averaging 4.1% collagen (total meat basis), compared to 0.29% in Atlantic salmon and 0.29-0.57% in beef (Olaechea and others 1993, Aidos and others 1999, Nakamura and others 2010). When compared to squid collagen contents, abalone is more similar with 20.5% collagen (total protein basis) compared to squid with 34%-42% collagen (total protein basis) (Nam and others 2008). Abalones have two distinct muscles, the adductor and the foot (Fallu 1991). The adductor connects the animal to its shell, while the foot is used for contraction, surface adherence, and mobility. Foot meat typically has higher collagen content than adductor meat, and is proportionally tougher (Olaechea and others 1993, Allen and others 2006).

Collagen content contributes the most to acceptance of abalone texture, and it varies with harvest season, environment, water temperature, diet, age, sex, and spawning status (Olaechea and others 1993, Hatae and others 1995, Allen and others 2006). Wild

abalones have not been shown to have significantly different collagen content compared to cultured abalones, despite the different environmental conditions such as currents and waves (Olaechea and others 1993, Wells and others 1998). The most drastic effect on collagen content is due to the time of year. Abalones harvested in the summer have significantly less collagen than abalones harvested in the winter (Olaechea and others 1993, Hatae and others 1995, Allen and others 2006). Hatae and others (1995) compared abalones harvested in July and November and found the July harvested abalones to be more tender with thicker, less compact collagen fibers, and greater water holding capacity compared to those harvested in November.

The total collagen as a percentage of muscle protein was reported to be 9.34% in July and 30.82% in November, suggesting that the abalones may store collagen as an energy source to be used during spawning. Younger, and proportionally smaller, abalones have been shown to have less collagen and to be more tender and acceptable to consumers than older, larger abalones (Olaechea and others 1993, Allen and others 2006). In addition to total collagen, the type of collagen can be used to characterize textural differences. Collagen can be identified as alkaline soluble, acid soluble, pepsin soluble, heat soluble, and insoluble. Heat solubility is important for understanding changes that take place during thermal processing. Characterization of abalone collagen solubility is lacking in the literature, though it has been shown in other aquatic species to significantly contribute to textural properties and functionality. Scallops have been shown to have less than 20% heat soluble collagen which contributes to structural stability during thermal processing (Mizuta and others 2007). High levels of alkaline insoluble collagen in salmon fillets have been correlated with increased firmness, thought

to be related to the amount of reducible crosslinks with collagen (Johnston and others 2006). Consumer acceptance of abalone meat has been consistently correlated with the quantity of total collagen, and processing efforts have been limited to thermal and mechanical processes to tenderize collagenous, tough meat (Olaechea and others 1993).

Abalone meat may be thermally tenderized by boiling, steaming, or canning. Cooking causes tenderization by two principal biophysical changes. The first is the denaturation and separation of myofibrils, which become less compact and easier to masticate (Gao and others 2002). The second is the conversion of collagen to gelatin, which contributes to the softer, chewier texture of cooked abalone meat compared to raw abalone meat (Gao and others 2001). Collagen is known to melt at 60°C in mammalian muscle, but has a significantly lower melting point in aquatic species, ranging from 27.4-33.6°C (Sikorski and others 1985, Cross 1986). The collagen in abalone has been reported to melt at 28°C, and this low melting point is believed to be due to the few gly-pro-ala and gly-pro-hyp sequences in the collagen fibers (Kimura and Kobota 1968). The lower gelatinization temperatures of aquatic animal collagen results in less intensive cooking requirements compared to land animals.

Cooking time, temperature, and medium have significant effects on abalone collagen gelatinization and meat tenderness. Long cooking times, up to 180 min, produce more tender and flavorful meat preferred by sensory panels compared to meat cooked for less time (Hatae and others 1996). Boiling abalone results in more tender meat than does steaming, which toughens abalone meat due to greater water loss and higher cooking temperatures increasing the viscosity of the meat (Gao and others 2002, Chiou and others 2004). Boiled abalone meat experienced a 38% loss of weight compared to raw abalone

meat, due to loss of water but also water-soluble flavor compounds (Gao and others 2001). Raw meat may be more flavorful compared to boiled or steamed meat due to water retention, but its toughness must be overcome for consumers who prefer more tender meat.

Mechanical tenderization is used routinely in the United States for beef, and in California raw abalone meat is frequently sliced, pounded gently, and sold fresh or frozen (Catalina Offshore Markets 2010, Giovanni's Fish Market and Gallery 2012). Mechanical tenderization separates collagen and muscle fibers, physically disrupting them and rendering the meat less tough (Tyszkiewicz and Jakubiec-Puka 1995). In beef, mechanical tenderization has been shown to be effective and the proportion of samples perceived by a sensory panel to have connective tissue decreased from 36% to 12% following mechanical tenderization (Jeremiah and others 1999). Mechanical tenderization of beef has been shown to increase thaw-drip and cooking losses compared to non-tenderized meat (Jeremiah and others 1999). Comparable studies with abalone have not been conducted. The physical disruption of abalone meat during mechanical tenderization may negatively affect the appearance of the whole steak if splitting occurs. Microbial cross-contamination of meat is also of concern when pounding one steak after another (Health Canada 2012). As such, alternative tenderization processes that tenderize meat without sacrificing raw meat qualities, such as water retention and flavor, are now being explored.

One such effort has been the use of food grade additives to tenderize meat. The proteolytic enzyme papain was investigated for the tenderization of abalone but the treatments did not have a significant effect on texture (Sanchez-Brambila and others

2002a). The high collagen content was believed to have prevented tenderization of the papain-treated abalone, however papain activity as well as solution temperature and volume may have played a role as well. Since the structure and surface area of collagen has been shown in beef to be affected by HPP, it is possible that HPP followed by a papain treatment would cause tenderization without the need for mechanical tenderization.

1.2. High Pressure Processing

High pressure processing (HPP) of foods was first investigated in the early 1900s, and explored extensively in the 1970s, but did not attract significant industry attention until the last decade. HPP is application of uniform high hydrostatic pressure to a food product which precludes bursting or crushing, however, many changes may occur at the cellular level. Some of these changes include enzyme inactivation (polyphenol oxidase (PPO) in particular), bacterial cell wall destruction (pasteurization), pigment destruction (bleaching), and protein deformation (tenderization) (Rönner 1995, Corwin and Shellhammer 2002, Ichinoseki and others 2006, Waite and others 2009, Del Olmo and others 2010, Perera and others 2010).

There are many advantages of HPP, including the maintenance of favorable product characteristics, such as vitamin profile, flavors and aromas (Rönner 1995, Hugas and others 2002). Additionally, HPP does not require heat or additives for effective shelf-life extension. Unlike in thermal processes where it takes time for the product to reach the same temperature as its environment, the application of pressure throughout the product is instantaneous. Heat or additives may be used in conjunction with HPP to

contribute to successful hurdle technology and product quality. The success of HPP in destroying bacteria and extending microbial shelf-life is due to its effect on the cell wall of the microorganisms within the product. This effect is based on the principle of Le Chatelier, which states that change in equilibrium within a system must be compensated with an opposite change to create a new equilibrium (Hugas and others 2002). HPP follows this principle whereby as pressure is increased (first change in equilibrium) the volume must decrease (second change in equilibrium). Once the pressure is returned to atmospheric pressure, the volume change is reversed to attain the original state of equilibrium. This rapid change in pressure (and thus volume) damages the integrity of the cell wall due to the compression and expansion of the cellular fluid during and after processing, respectively. Additional effects due to the pressure itself, and not specifically the change in pressure, include reduction in cell membrane function through changes in hydrogen bonds, electrostatic and hydrophobic interactions, and protein structure which leads to denaturation (Rönner 1995).

Most processing pressures used in the food industry range between 100-600 megapascals (MPa), or 14,500 to 87,000 pounds per square inch (PSI), which is sufficient to extend shelf-life (Hugas and others 2002). A pressure of 300 MPa has been shown to be effective against Gram-negative bacteria, such as *Salmonella spp., Escherichia spp.*, and *Vibrio vulnificus*, while 600 MPa is required to destroy Gram-positive bacteria, such as *Clostridium spp., Bacillus spp., Listeria monocytogenes,* and *Staphylococcus spp.* (Rönner 1995, Kural and Chen 2008, Porto-Fett and others 2010). The difference in effect on Gram-negative compared to Gram-positive bacteria may be due to the thicker layer of peptidoglycan in Gram-positive bacteria, which may provide some level of

resistance against cell wall destruction by HPP due to the covalent bonds between the peptidoglycan molecules. Covalent bonds do not appear to be affected by HPP the way hydrogen and hydrophobic bonds are (Cheftel 1995).

Microorganisms that are acclimated to cold temperatures are more resistant to HPP due to greater membrane fluidity and less susceptibility to the volume changes associated with HPP (Hugas and others 2002). The shape of the microorganism also affects the efficacy of HPP, with cocci being more resistant than rods. Spore inactivation, however, requires substantially greater pressures combined with heat, which may negate some of the benefits of HPP as a mild processing treatment. *Bacillus* spores have been shown to be destroyed during HPP when held at 71°C for 16.8 min at 540 MPa, but destruction of *Clostridium botulinum* spores required 620 MPa at 100°C for 10 min or 827 MPa at 75°C for 20-30 min (Reddy and others 2006, Gao and Ju 2010). Destruction of yeasts and molds only required 400 MPa by comparison, unless it was a high moisture product, in which case 200 MPa was effective.

1.2.1. Industrial Applications

HPP has been shown to be useful for shelf-life extension of many different food products, such as fruit, dressings, cheese, meat, and seafood (Rönner 1995, He and others 2002, Hugas and others 2002, Yagiz and others 2007, Waite and others 2009, Okpala and others 2010, Perera and others 2010). As demand for fresh, natural, preservative-free products increases, interest and awareness of HPP will likely increase correspondingly. There has been much commercialization of HPP in the meat industry due to its preservation of natural flavors and fresh appearance of raw and cured meat without the

use of preservatives (Hugas and others 2002). Fresh, post-rigor beef exhibited refrigerated shelf-life extension of up to 42 days following HPP at 310-345 MPa, though lower pressures did not result in the same effect (Sánchez-Basurto and others 2011). Fresh and cured pork had extended shelf-life after treatment at 600 MPa for 10 min (Hugas and others 2002).

Texture is probably the second most important parameter, after food safety, to consider when evaluating the efficacy of HPP treatment. HPP has been shown to toughen meat, such as chicken (400 MPa) and beef (500 MPa), with longer processing times (3-20 min compared to 1 min) resulting in tougher meat (Del Olmo and others 2010, Duranton and others 2012). It is believed that meat toughening due to HPP is due in part to myofibrillar protein denaturation and myosin compression, while tenderization at pressures less than 400 MPa may be due to the release of calcium ions as well as proteases (Zamri and others 2006). Collagen content appears to be inversely proportional to meat tenderness and while HPP does not directly improve tenderness of collagen, it was shown to increase the heat solubility of collagen suggesting potential for improved tenderness of HPP meat after cooking (Ichinoseki and others 2006).

Rigor status of muscle during processing has also been shown to affect tenderization of HPP meat. Living muscle experiences alternating patterns of contraction and relaxation dependent on the presence of adenosine triphosphate (ATP). Upon death, oxygen depletion causes cells to revert to anaerobic glycolysis which does not produce ATP at levels required to continue the contraction and relaxation cycle of muscle (Strasburg and others 2008). As a result, the muscle remains fully contracted, termed rigor, through a fusion of actin and myosin that is eventually released enzymatically. The

relaxation of the meat is termed resolution and its onset varies depending on species, glycogen content before death, and temperature. The effect of abalone rigor status during HPP on meat quality has not been explored.

HPP also has a significant effect on color of meat, with greater pressures inducing a cooked meat appearance and general lightening of the product pigments. In red meat, ferrous myoglobin is converted to ferric myoglobin (>400 MPa) and globin is denatured (>400 MPa), both of which lead to increased whiteness values as evidenced in chicken and beef (Hugas and others 2002, Del Olmo and others 2010, Sánchez-Basurto and others 2011). Freezing of beef prior to HPP has been shown to prevent color degradation, though the specific mechanisms of color preservation due to freezing are unknown (Fernández and others 2007). Other biochemical changes that occur in meat due to HPP include calpastatin inhibition (>200 MPa), calpain (>400 MPa) and lysosome degradation (<200 MPa), and inactiviation of cathepsin B, H, and L (>500 MPa), cathepsin D (>300 MPa), and aminopeptidase (>200 MPa) (Hugas and others 2002, Chéret and others 2005, Chéret and others 2007). HPP, like many processes, has different effects on different products and requires careful evaluation to determine product-specific parameters based on desired quality outcomes. The product itself is a variable, in addition to pressure, time, and temperature, and so HPP is a product-specific process.

As such, HPP parameters that have been found to be effective for beef and pork may be too extreme for seafood products, which have a much different composition. There must also be a balance between the higher pressures and temperatures required for shelf-life extension (spoilage organisms) or food safety (pathogens and spores) and maintenance of food quality and consumer acceptance. Generally, lower pressures are

associated with higher quality of the final product with regard to color, texture, and water-holding capacity. The food industry is thus charged with finding the minimum processing parameters to achieve safe, long-lasting, high quality products. The specificity of HPP application, however, makes it difficult to make sweeping generalizations and so discussion of particular product types is necessary.

1.2.2. High Pressure Processing of Seafood

The more delicate nature of fish muscle and the higher concentrations of polyunsaturated fatty acids (PUFA) compared to land animals, such as beef and pork, promote the use of lower pressures in order to preserve its texture and sensory properties. During HPP, the disruption of cell walls and potential release of metal ions and heme proteins may be the cause of increased lipid oxidation with increased pressure, but this is highly dependent on fish species (Yagiz and others 2007). Mahi mahi and Atlantic salmon, for instance, showed decreased lipid oxidation at higher pressures (450 MPa and 300 MPa, respectively) compared to lower pressures (300 and 150 MPa, respectively), but rainbow trout showed increased lipid oxidation above 300 MPa (Yagiz and others 2007, Yagiz and others 2009). Shelf-life extension studies for finfish have been minimal, with 3 day extensions possible for fish such as red mullet (330 MPa) but no extensions for salmon processed at 135, 170, and 220 MPa (Briones and others 2010, Erkan and others 2010).

By contrast, oysters subjected to moderate HPP of only 350 MPa may experience shelf-life extensions of up to 21 days, and also demonstrate a greater than 5 log reduction in the pathogen *Vibrio vulnificus* at pressures of at least 250 MPa (Kural and Chen 2008,

Lai and others 2010). Shrimp and clams processed at 220 and 250 MPa, respectively, were shown to have a shelf-life extension of 12-14 days, respectively, compared to non-HPP controls (Büyükcan and others 2009). Abalones experienced a shelf-life extension of 30 to 35 days compared to controls after processing at 500 and 550 MPa (Briones and others 2010, Briones-Labarca and others 2012). These studies demonstrate the relationship between higher pressures and longer shelf-life, and the importance of determining whether the loss in overt quality, such as color and texture, is worth the additional days of storage. In addition to shelf-life extension of shellfish, HPP is used frequently in the seafood industry to shuck bivalves and crustaceans, such as oysters and lobsters. Investigation into the ability of HPP to improve shucking of single-shelled mollusks such as abalones has yet to be documented.

Regarding seafood texture and color following HPP, there was not a significant effect of pressure on the firmness of treated abalones (500 or 550 MPa) compared to controls (Briones-Labarca and others 2012). In the same study, higher pressure (550 MPa) for longer times (8 min) caused lower whiteness values compared to controls, however it has been shown that whiteness and opacity increases with increased pressure for mackerel, cod, bluefish, carp, trout, salmon, and red mullett (Ohshima and others 1993, Angsupanich and Ledward 1998, Master and others 2000, Sequeria-Munoz and others 2006, Yagiz and others 2007, Yagiz and others 2009, Erkan and others 2010). Comparative studies of the effect of HPP on abalone meat color is lacking in the literature. Whitening would be a desirable effect of HPP since whiter abalone meat is more desirable to consumers but can be hard to achieve using algal diets resulting in the

need for trimming or chemical bleaching of the meat (Oakes and Ponte 1996, Allen and others 2006, Brown and others 2008).

1.3. Conclusions

High pressure processing (HPP) appears to be a promising technology to minimally process abalone meat. Clear potential advantages include the ability to extend the shelf-life of raw meat and to increase its whiteness without the use of additives. The lack of a raw shucked market for abalones presents a unique opportunity to deliver HPP abalone meat in its most desirable form without the burden of live shipment and subsequently short shelf-life. Tenderization of the abalone steak through HPP and papain would offer another product with potentially higher acceptance than mechanically or thermally tenderized meat. As a high value delicacy, consumers and chefs may be willing to pay a premium for HPP abalones that reduce labor and improve consumer acceptance.

1.4. Objectives

The objectives of this research were to evaluate the effects of high pressure processing on abalone meat texture, color, shelf-life, and susceptibility to papain tenderization. Research into a process such as HPP that provides shucked raw abalone meat to consumers and chefs may provide new opportunities to aquaculture and shellfish industries at the same time. The specific objectives were to evaluate the effects of 1) rigor status during HPP on abalone quality, 2) HPP on subsequently cooked abalone, 3) processing pressures of <500 MPa on shelf-life, and 4) HPP followed by papain treatment on physicochemical qualities of abalone.

CHAPTER 2

EFFECT OF RIGOR STATUS DURING HIGH PRESSURE PROCESSING ON THE QUALITY OF FARM-RAISED ABALONE (*Haliotis rufescens*)

2.1. Objectives

High pressure processing (HPP) is known to affect different changes on mammalian meat quality depending on whether the meat is pre-rigor or post-rigor during HPP. It has been well-documented for beef that pre-rigor processing results in more tender meat compared to unprocessed beef and post-rigor processed beef, unless high temperatures are used in conjunction with HPP (MacFarlane 1973, Bouton and others 1977, Kennick and others 1980, Jung and others 2000, Sikes and others 2010). The effect of rigor status on seafood quality is infrequently discussed except as it pertains to gaping, or muscle segment separation, in fish such as cod and salmon (Lauritzsen and others 2004, Esaiassen and others 2008, Larsen and others 2008). Evaluations of rigor status of shellfish such as lobster and scallop are very limited (Gornik and others 2009, Jiménez-Ruiz and others 2013), and have not been reported for HPP abalone. The objectives of this study were to assess the effects of 1) abalone rigor status during HPP on texture and color of raw abalone, 2) different HPP parameters (pressure and time) on texture and color of raw abalone, and 3) HPP on texture and color of different abalone muscles (adductor and foot).

2.2. Materials and Methods

The HPP study had a 2x2x3 multifactorial design, with rigor status (pre- and post-rigor), pressure (100 and 300 MPa), and time (1, 3, 5 min) as the treatment variables (Table 2.1). Post-rigor unprocessed meats were used as the control since all meats were post-rigor at the time of analyses. Samples were coded according to rigor status (PRE or POST), time (1, 3, or 5 min) then pressure (100 or 300 MPa), for example, PRE-5-300 was processed pre-rigor for 5 min at 300 MPa. Abalones were processed whole, but were divided into adductor and foot for all analyses (moisture, protein, collagen, color, texture, and scanning electron microscopy).

Each of the 12 processing treatments (plus the unprocessed control treatment) contained 12 abalones (n=156), with an additional 12 abalones reserved to serve as a pre-rigor control for a rigor comparison study, three unprocessed abalones reserved for collagen analyses, three live unshucked abalones for an observational shucking evaluation, and three whole shucked abalones for an observational shelf-life evaluation, for a total of 177 abalones. Reagents were analytical grade and were purchased from Fisher Scientific (Waltham, MA) unless otherwise noted. Samples for collagen analyses were frozen after processing. Texture, color, and moisture analyses were conducted within three days of processing. Scanning electron microscopy (SEM) and protein samples were prepared within three weeks of processing. Values are presented on a wet-weight basis (wwb).

Table 2.1. Experimental design of rigor status study.

Processing Parameters	100 MPa	300 MPa
1 min	1,100	1,300
3 min	3,100	3,300
5 min	5,100	5,300
Muscle Analyzed	Adductor or Foot	Adductor or Foot
Rigor Status during Processing	Pre-rigor (PRE) or Post-rigor (POST)	PRE or POST

2.2.1. Rigor Status Evaluation

A guideline of time to rigor and rigor resolution was needed to inform the pre-rigor and post-rigor processing schedule. Whole abalones (n=2) (The Abalone Farm, Cayucas, CA) were shucked by hand, eviscerated and placed on ice at 2°C. The abalones were assessed by compression force (N), foot side up, initially, at 6 h, at 12 h, and every 2 h from then until rigor resolution using a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY). Force (Newtons, N) was recorded by the texture analysis software (Exponent 32, version 5,0,6,0, 2010, Texture Technologies Inc., Scarsdale, NY). A 10 mm cylinder Delrin P/10 probe was used at a pre-test speed of 5 mm/s, a test speed of 2 mm/s, and a post-test speed of 10 mm/s. The target mode was distance, set at 5 mm. The trigger type was force, set at 0.04903 N. To evaluate the effect of rigor status on unprocessed abalone texture and color, 12 abalones were shucked only 1 hr prior to color and texture analyses (described subsequently) to ensure the abalone meat was pre-rigor. The color and texture results were then compared directly to those of the post-rigor control as a means of directly comparing the effect of rigor status exclusive of processing.

2.2.2. Processing

Live, July-harvested, farm-raised abalones (*Haliotis rufescens*) (n=117) (The Abalone Farm, Cayucas, CA) were divided according to the experimental design. Individual abalones were weighed and measured. The mean weight (n=40) of the in-shell abalones was 88.9 g ± 11.5; the mean shell length was 82.7 mm ± 6.7. Abalones had not been fed for a minimum of 5 days prior to shucking. Abalones were shucked, eviscerated, packed at 99% vacuum (Model UV550, Koch Industries, Wichita, KS) (six per bag), and stored on ice at 2°C until processing. POST abalones, including control, were stored on ice for at least 40 h after shucking and PRE were stored on ice for between 1 and 6 h after shucking. Treatments were randomly processed using a 1 L HPP unit (Engineering Pressure Systems Inc., Haverhill, MA). The temperature of the vessel during pressurization ranged from 23°C-28°C. Hydraulic fluid (20:1 water:Hydrolubric 120-B (Houghton International Inc., Norristown, PA)) was used to achieve hydrostatic pressure. The come-up time ranged from 3 min to 4.5 min and depressurization was immediate. The 12 abalones reserved for the rigor comparison study were kept alive until 1 h prior to analyses to ensure they were pre-rigor during analyses.

2.2.3. Moisture and Protein Content

Moisture content was determined gravimetrically by drying duplicate 3 g samples of chopped unprocessed abalone adductor (n=3) and foot (n=3) overnight in a 105°C oven (Fisher Isotemp, Barrington, IL) (AOAC 2005). Protein content was determined in duplicate on previously dried unprocessed abalone adductor (n=3) and foot (n=3) with a combustion nitrogen analyzer (Rapid N III, Elementar Americas Inc, Mount Laurel, NJ).

Aspartic acid (Sigma-Aldrich, St. Louis, MO) was used as the nitrogen standard, and a conversion factor of 6.25 was used to determine crude protein content. Both moisture and protein contents are reported in g/100 g (wwb).

2.2.4. Collagen Content

Collagen may be qualified as the total amount of collagen in g/100 g meat, or by its solubility in acid and pepsin. Total collagen was evaluated following the AOAC method (AOAC 1995) for hydroxyproline in meat. Abalones (n=3) had been frozen at -12°C for 4 months prior to collagen analysis. Abalones were thawed overnight at 4°C and divided into adductor and foot. The samples were chopped and 4 g of each were added to 30 mL 7 N H_2SO_4. Gelatin (Knox, Deerfield, IL) was used as a control and 0.5 g was added to 30 mL H_2SO_4 for hydrolysis. Acidified samples were placed in a 105°C oven (Fisher Isotemp, Barrington, IL), for 16 h, cooled, and quantitatively brought to 500 mL with deionized (DI) water. Solutions were filtered and stored at 4°C for a maximum of 2 weeks. Final dilution of the solutions were obtained by bringing 2 mL solution (1 mL for gelatin controls) to 100 mL with DI water, and then using 2 mL of that concentration directly for spectrophotometric quantification.

Acid and pepsin soluble collagen fractions were extracted following established methods (Sato and others 1988, Espe and others 2004). For the alkali pre-extraction, 2.5 g abalone was microhomogenized for 30 s (Polytron, Brinkmann, Westbury, NY) in 10 volumes 0.1 M NaOH. The mixture was centrifuged (model J2-21, Beckman Coulter, Brea, CA) at 10,000 *g* for 20 min and the supernatant discarded. To the pellet, 20 volumes 0.1 M NaOH were added and the solution was vortexed before being placed on a

shake plate (model 1314, Labline Instruments, Melrose Park, IL) at 4°C for 12 hours. The latter alkali extraction was repeated 3 more times. The final pellet was washed by immersing it in DI water, vortexing, and re-centrifuging at 10,000 *g* for 20 min prior to acid soluble collagen (ASC) extraction.

The water-washed alkali pellets were vortexed with 10 volumes 0.5 M acetic acid and left on a shake plate at 4°C for 2 days. The solution was centrifuged at 10,000 *g* for 20 min and the supernatant volume was quantified and retained as the ASC fraction. For the pepsin soluble collagen (PSC) extraction, the acid pellet was vortexed with a 20:1 ratio of original sample mass:pepsin (porcine, 400 units/mg, Sigma-Aldrich, St. Louis, MO) and 10 volumes 0.5 M acetic acid, then placed on a shake plate at 4°C for 2 days. The solution was centrifuged at 10,000 *g* for 20 min and the supernatant volume was quantified and retained as the PSC fraction. The pellet was retained as the insoluble collagen (ISC) fraction.

Hydrolysis of the soluble collagen fractions was completed by adding 1 mL 60% H_2SO_4 to 1 mL of each soluble collagen fraction and the entire ISC pellet, then placing them in a 105°C oven for 12 h. Hydrolyzed samples were neutralized to pH 6-7 with 5 N NaOH. The ASC fraction was brought to 10 mL with DI water, and 2 mL were used directly for spectrophotometric quantification. The PSC fraction was brought to 100 mL then was further diluted by adding 1 mL ISC dilution to 1 mL DI water for quantification. The ISC fraction was brought to 100 mL with DI water, and 10 mL of that dilution was brought to 200 mL with DI water. The hydroxyproline (Sigma-Aldrich, St. Louis, MO) standard curve was made fresh daily. The chloramine-T (Sigma-Aldrich, St. Louis, MO) oxidant solution, 4-dimethylaminobenzaldehyde (Sigma-Aldrich, St. Louis, MO) color

reagent, and acetate-citrate buffer were made as described in the AOAC method (AOAC 1995). To each 2 mL aliquot of final dilution was added 1 mL oxidant solution, then it was vortexed and allowed to stand at room temperature for 20 min. The color reagent, 1 mL, was then added, vortexed, and immediately immersed in a water bath maintained at 60°C for exactly 15 min, cooled, and absorbance was read in a spectrophotometer (DU530, Beckman Coulter, Brea, CA) at 558 nm. Hydroxyproline concentration (g/100 g) was determined by comparing sample absorbance to the hydroxyproline standard curve. The collagen content (g/100 g) was calculated using a conversion factor of 9.8 since abalone collagen is reported to contain 10.2% hydroxyproline (Kimura and Kubota 1968).

2.2.5. Colorimetric and Texture Analyses

Treatments were analyzed in random order. The whole abalones were sliced horizontally below the base of the adductor to separate the foot from the adductor muscle. Square plugs, one per abalone, measuring 20x20x7 mm by a digital caliper were cut from the center of each muscle. Plugs from the foot were cut from the ventral side, and plugs from the adductor were cut from the dorsal side. Plugs were stored in 7.5 x 12.5 cm WhirlPak (Nasco, Fort Atkinson, WI) bags at 4°C until analyses.

2.2.5.1. Color

Colorimetric analyses (n=12) were performed using a colorimeter (LabScan XE, Hunter Labs, Reston, VA). The Hunter L, a, b values were recorded by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs, Reston, VA). The colorimeter was standardized using white and black tiles for a port size of 17 mm and an area view of 25

mm. Plugs were positioned on a watch glass above the colorimeter, and after the initial color reading, the watch glass was rotated 120º. The rotation was repeated a final time to achieve three readings, which were then averaged to one value per plug.

2.2.5.2. Texture

For texture analyses, the twelve abalones per treatment were divided in half to conduct two different texture analyses: texture profile analysis (TPA) and shear. Following color analysis, individual plugs were placed on the calibrated texture analyzer platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) non-cut surface side up. For TPA (n=6), the texture analyzer was configured with a 10 mm probe, 2 mm/s test speed, and a 5 s gap between compressions. TPA emulates chewing, and can be useful for comparison to the consumer experience (Bourne 2002). Force (Newtons, N), area (N*s), and time (s) were recorded by the texture analysis software (Exponent 32, version 5,0,6,0 2010, Texture Technologies Inc., Scarsdale, NY) to calculate TPA parameters.

The four parameters evaluated included firmness, springiness, resilience, and chewiness. Firmness is resistance to compression, calculated by the peak force (N) of the first compression (Bourne 2002). Springiness indicates how well a product springs back after compression, a unitless number calculated by dividing the distance to peak force of the second compression by the distance to the peak force of the first compression (Bourne 2002). Resilience describes the immediate springiness as the probe is withdrawn between "bites", a unitless number calculated by dividing the area of the withdrawal of the first compression by the area of the first compression (Bourne 2002). Chewiness characterizes elastic resistance of a solid food, a unitless number calculated by

multiplying firmness, cohesiveness (resistance to a second compression compared to the first), and springiness (Bourne 2002). The plugs (n=6) for shear force analysis were placed so the blade (A/CKB craft knife blade) cut across the muscle fibers. The texture analyzer was configured to a 6 mm depth and a 5 mm/s test speed. Units for both TPA and shear force were given in N, except for the TPA attributes springiness, resilience, and chewiness which are unitless. Samples were kept in ice slurry prior to evaluations.

2.2.6. Scanning Electron Microscopy

Abalone samples were prepared for microwave enhanced fixation by using a razor blade to slice pieces (n=3) no thicker than 1 mm from the center of the muscle. Foot and adductor muscles from control, minimum (1-100), and maximum (5-300) HPP treatments were selected to optimize observation of potential visual treatment effects. Stock 0.2 M phosphate buffer was made by combining 0.22 g $NaH_2PO_4 \cdot H_2O$, 1.19 g Na_2HPO_4, and 8 g sucrose in 50 mL deionized water to a final pH of 7.3. Working buffer was made by making a 1:1 ratio of stock phosphate buffer:deionized water to a final concentration of 0.1 M, with the addition of 1 drop 1% $CaCl_2$ per 10 mL buffer. Specimens were fixed in 2.5% glutaraldehyde (Electron Microscopy Services, Hatfield, PA), made by combining 3 mL 25% glutaraldehyde, 12.5 mL stock buffer, and 2 drops 1% $CaCl_2$. The solution of 1% osmium tetroxide (OsO_4) (Electron Microscopy Services, Hatfield, PA) was prepared by combining 2% OsO_4 in equal parts stock buffer.

Iced specimens, in vials, were fixed in approximately 2 mL 2.5% glutaraldehyde solution in a pre-warmed microwave oven for 1 7 s cycle on high, followed by a 20 s rest, and a final 7 s cycle on high (7/20/7). The initial fixation was followed by a replacement

of fixative with working buffer and a 7/20/7 cycle. The buffer rinse was repeated once for a total of two rinses. The rinsed specimen was then fixed with 1% OsO_4 for 1 7/20/7 cycle and rinsed with deionized water. Fixed specimens were dehydrated in serially increasing concentrations of ethanol (50%, 70%, 95%) for two 7/20/7 cycles at each level. Following the 95% dehydration, samples were further dehydrated in 100% ethanol three times for 7 min each time at room temperature. The samples were stored in 100% ethanol until critical point drying.

Critical point drying (Samdri PVT-3, Tousimis Research Corp., Rockville, MD) was achieved in a pre-cooled specimen chamber in 100% ethanol which was gradually replaced with liquid carbon dioxide in five 5 min soaks. After the last soak, the chamber was 75% filled with liquid carbon dioxide and the heater was turned on until the chamber reached 45°C and pressure no greater than 1400 psi. The chamber was very slowly exhausted and the dried samples were transferred to stubs affixed with carbon-coated tape and silver adhesive (503, Electron Microscopy Sciences, Hatfield, PA). The samples were sputter coated (Cressington 108 Auto, Redding, CA) at 40 mA and 0.08 mbar for 90 s to generate a 35 nm layer of gold-palladium on the surface. Samples were stored in a dessicator until imaging. The scanning electron microscope (AMRay 1820 Digital SEM, Bedford, MA) was degaussed initially and between samples. An accelerating potential of 10 kV and a spotsize of 10 were selected, and magnification up to 2,000 times (2000x) was used. Foot myofibril widths (n=10) were measured in each image and averages were used for statistical analyses.

2.2.7. Shucking Observational Study

Live unshucked abalones (n=3) were individually packed at 99% vacuum (Model UV550, Koch Industries, Wichita, KS) and two were processed simultaneously for 5 min at 300 MPa, with one reserved as a control. Following processing the abalones were qualitatively evaluated by the author for ease of removal from the shell by hand without utensils.

2.2.8. Microbiological Observational Study

Whole shucked abalones (n=3) were aseptically placed in individual filtered stomacher bags with sterile 0.1% bactopeptone (BD Diagnostics, Sparks, MD) (1:10 w/w ratio). There were two unprocessed control abalones and one abalone processed for 1 min at 100 MPa. The bags were mixed for 2 min using a BagMixer 400 (Model P, SpiralBiotech, Advanced Instruments, Norwood, MA). Serial dilutions of 1:10, 1:100, and 1:1000 were used to quantify aerobic bacterial loads. Aliquots of 1 mL of each dilution were dispensed on Aerobic Plate Count PetriFilm (3M, St. Paul, MN) in duplicate and incubated for 2 days at 35°C. Averages from the duplicate plates were used to compare results.

2.2.9. Statistical Analyses

Data were analyzed using SYSTAT 12 (Systat Software, Chicago, IL) for one-way analysis of variance (ANOVA) for all one-level (treatment) analyses, and multi-way ANOVA to assess multiple independent variables. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses except where Tukey's was too conservative to find differences identified by ANOVA, in which case Fisher's Least

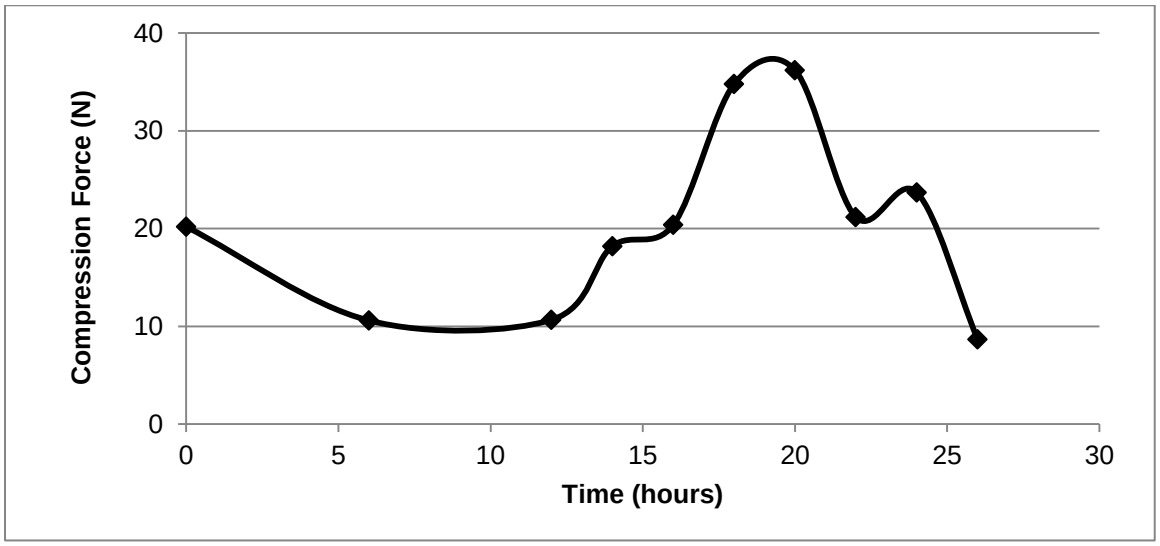
Significant Difference (LSD) test was used. Shapiro-Wilk test was used to assess normality and Levene's equality of variances test was used to assess homogeneity. In cases where data did not satisfy either normality or homogeneity, they were evaluated non-parametrically using Kruskal-Wallis. Mann-Whitney was selected for non-parametric post-hoc analyses. For all statistics, a significance level of $p<0.05$ was selected.

2.3. Results and Discussion

2.3.1. Rigor Status Assessment

The results of the rigor study demonstrated that abalones were in a pre-rigor state until 12 h post-shucking, at which point the muscle required an increase in compression force (Figure 2.1). The time from rigor onset to resolution is dependent on the species, pre-mortem stress, feeding status at slaughter, and storage temperature (Mørkøre and others 2008). Time zero compression force was likely higher than the six hour time point because of contraction during shucking. Rigor was maximized between 16 and 20 h with an average compression force of 36.2 N compared to the average pre-rigor compression force of 10.6 N. Rigor resolution was achieved by 26 h, with average final compression force of 8.7 N. The onset of rigor occurs after ATP levels decline, with correlations between fast onset and low initial ATP levels due to starvation or pre-slaughter stress having been reported for salmon muscle (Mørkøre and others 2008). No comparable rigor studies have been published for abalone muscle.

Figure 2.1. Average compression force of abalone muscle during rigor evaluation (n=2).



To evaluate the effect of rigor status alone, pre-rigor unprocessed abalone meat was compared to the post-rigor unprocessed control (Table 2.2). Post-rigor foot meat had significantly higher L and b values than pre-rigor foot meat, but there were no significant differences for a values. The increase in L and b values of post-rigor unprocessed foot meat suggests that during rigor resolution some of the pigments changed, perhaps due to proteolysis, causing universal post-rigor lightening of muscle color. The adductor, already very light in color, did not have any significant changes in L or a values, though b values did decrease in post-rigor adductor.

Shear force was higher for post-rigor foot, and lower for post-rigor adductor, which was in contrast with TPA results. TPA demonstrated a remarkable reduction in firmness and chewiness from pre-rigor to post-rigor for both foot and adductor, though the foot was 7 times firmer than the adductor whether pre-rigor or post-rigor. The two texture analyses assess different aspects of meat texture, with shear demonstrating the resistance of collagen to breaking stress compared to muscular compressibility (Olaechea

and others 1993). Springiness was similar for both foot and adductor, increasing in post-rigor treatments. Resilience was the opposite and decreased in post-rigor treatments for both adductor and foot. Overall it appears that more tender foot meat can be achieved by holding abalone meat through rigor resolution as opposed to consuming it immediately after shucking. The increase in L values in particular may benefit retailers selling the meat to consumers who prefer whiter meat.

Table 2.2. Pre-rigor and post-rigor comparison of unprocessed abalone color and texture.

	Foot		Adductor	
	Pre-rigor	**Post-rigor**	**Pre-rigor**	**Post-rigor**
L	48.3 ± 5.1 b	62.5 ± 6.2 a	84.2 ± 2.8 a	84.1 ± 4.7 a
a	12.0 ± 2.6 a	12.0 ± 2.6 a	4.1 ± 1.9 a	2.7 ± 1.2 a
b	18.6 ± 4.1 b	23.0 ± 1.8 a	14.7 ± 1.4 a	10.8 ± 1.4 b
Shear (N)	30.0 ± 3.7 b	39.8 ± 5.6 a	35.4 ± 3.4 a	24.3 ± 3.8 b
Firmness (N)	28.5 ± 7.5 a	7.7 ± 2.1 b	3.8 ± 2.0 a	1.8 ± 0.9 a
Chewiness	12.9 ± 4.5 a	3.2 ± 1.4 b	1.0 ± 0.2 a	1.1 ± 0.5 a
Springiness	0.8 ± 0.1 b	0.9 ± 0.1 a	0.7 ± 0.1 b	0.9 ± 0.1 a
Resilience	0.5 ± 0.1 a	0.3 ± 0.1 b	0.2 ± 0.0 a	0.3 ± 0.0 a

Each value is the mean ± standard deviation ($n \geq 6$). Values within rows, by muscle type, not sharing a lowercase letter are significantly ($p<0.05$) different, analyzed by ANOVA (Tukey's HSD post-hoc test).

2.3.2. Moisture and Protein Content

The moisture content of the foot was 75.0 g/100 g ± 0.8. The adductor contained 75.7 g/100 g ± 0.3 moisture which was not significantly ($p>0.05$) different from the foot. Protein content of the adductor was 17.5 g/100 g ± 0.2 (wwb), which was significantly lower than the protein content of the foot, 18.0 g/100 g ± 0.0 (wwb). These values are similar to the 73.9 g/100 g moisture and 21.6 g/100 g protein reported for whole farmed red abalone in Chile (Briones-Labarca and others 2012).

2.3.3. Collagen Content

It was expected that the foot would contain significantly more collagen than the adductor, however significant differences were not observed for total or pepsin soluble collagen (PSC) (Table 2.3). The foot contained 3.545 g total collagen/100 g meat and the adductor contained 3.668 g total collagen/100 g meat. Both muscles contained nearly 0.8 g/100 g PSC, but the adductor contained significantly more acid soluble collagen (ASC) at 0.013 g/100 g meat compared to the 0.007 g/100 g meat for the foot. Insoluble collagen (ISC) was significantly higher in the adductor as well, with 1.163 g/100 g meat in the adductor compared to 1.008 g/100 g meat in the foot. Due to the initial alkali wash, alkali soluble collagen is presumed to make up the difference between total collagen and the cumulative total of ASC, PSC, and ISC. ASC, PSC, and ISC values have not been previously reported for abalone.

Abalones harvested in summer typically have less collagen than those harvested in the winter, possibly explaining the lack of difference between adductor and foot. Collagen contents of farmed abalone (in-shell weight: 483 g) have been reported to be as high as 7.8 g/100 g meat for the foot and 3.06 g/100 g for the adductor (Olaechea and others 1993). The substantially lower foot collagen content reported in the current study may be due to the species (*Haliotis rufescens* compared to *H. discus*), time of year the farmed abalones were harvested (July compared to year-round), and size/age of the abalones (88.9 g compared to 483 g). Collagen content is known to vary widely depending on the above factors, and in another study adductor collagen was reported to be 2.26 g/100 g of the total meat and foot collagen was reported to be 3.32 g/100 g, though the specific information about the evaluated specimen was not discussed (Olley

and Thrower 1977). The very small size of these summer harvested farmed abalones may explain the significantly lower collagen content compared to other reports.

Table 2.3. Collagen content (g/100 g meat) of foot and adductor.

	Foot	Adductor
Total	3.545 ± 0.180 a	3.668 ± 0.223 a
Acid Soluble	0.007 ± 0.002 b	0.013 ± 0.002 a
Pepsin Soluble	0.794 ± 0.121 a	0.772 ± 0.026 a
Insoluble	1.008 ± 0.072 b	1.163 ± 0.048 a

Each value is the mean ± standard deviation (n=3). Values within rows not sharing a lowercase letter are significantly ($p<0.05$) different. All treatments were analyzed by ANOVA (Tukey's HSD post-hoc).

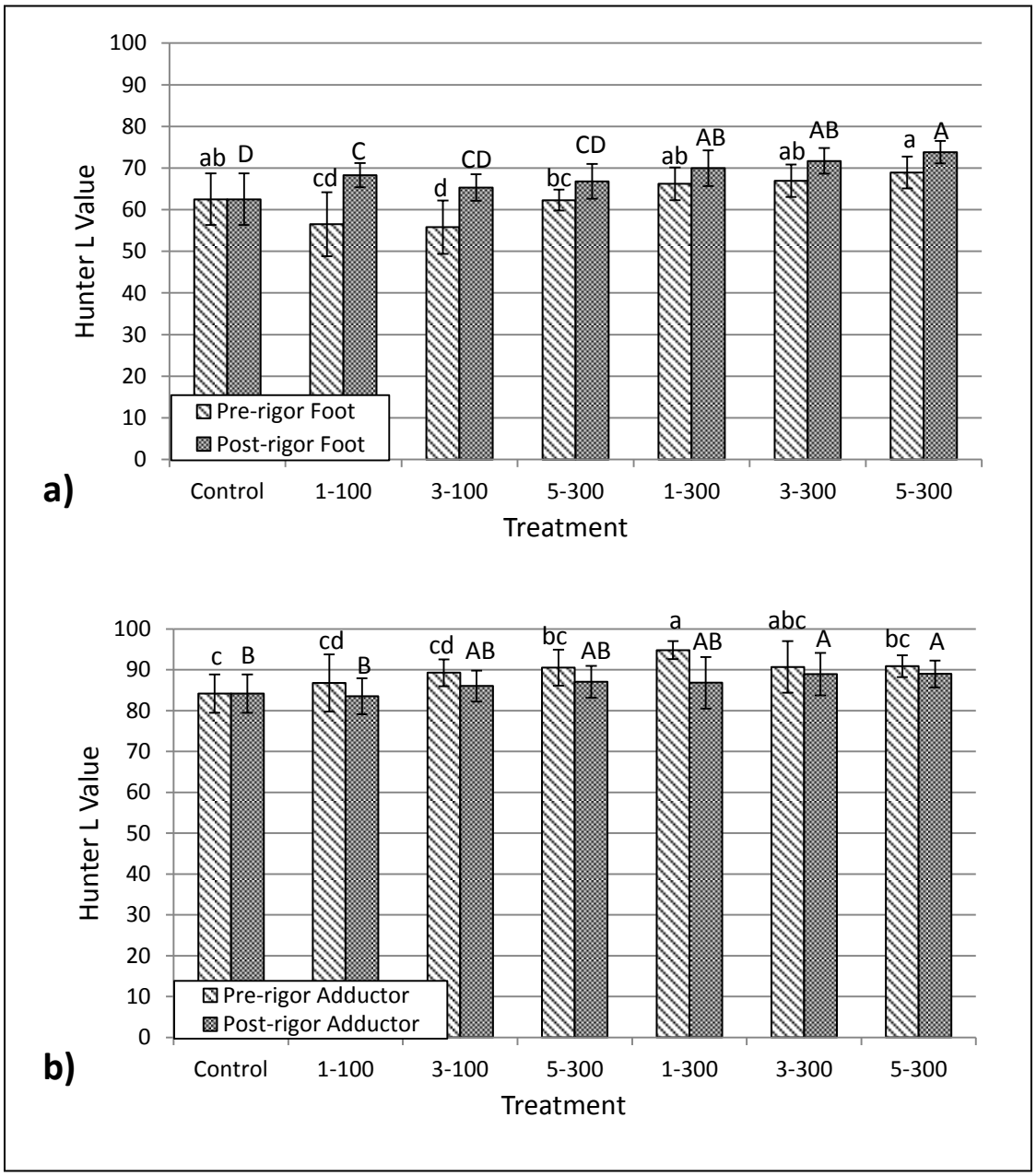
2.3.4. Color

The abalone foot muscle is known to be darker in color than the adductor due to the presence of pigments such as melanin, carotenoids, and bilichromes such as haliotisrubin (Voltzow 1994, Portela and others 2012). Consumers typically prefer lighter meat, and abalones with dark pigmentation may be heavily trimmed or bleached (Brown and others 2008). HPP has been reported to increase L and b values and decrease a values in trout, mahi mahi, red mullet, and salmon muscle, presumably due to denaturation of proteins such as myoglobin as well as oxidation of carotenoids and ferrous myoglobin to ferric metmyoglobin (Yagiz and others 2007, Yagiz and others 2009, Erkan and others 2010, Ojagh and others 2011). Similarly, HPP oyster meats have been shown to have increased L values, decreased a values, and unchanged b values compared to unprocessed controls (Lai and others 2010).

Foot and adductor color increased in L value with increasing pressure as well as with post-rigor processing (Figure 2.2). The HPP-induced increase in L value

(lightening) was most notable in the darker pigmented foot muscle. Foot L values increased from 62.5 (control) to 68.9 (PRE-5-300) and 73.8 (POST-5-300), though the increase was only significant for POST-5-300. Post-rigor processed foot had significantly higher values than pre-rigor processed foot, and L values increased with increasing pressure. Adductor L values significantly increased from 84.1 (control) to 90.9 (PRE-5-300) and 88.97 (POST-5-300).

Figure 2.2. Hunter L values. Abalone foot (a) and adductor (b) meat.



Each value is the mean ± standard deviation ($n \geq 8$). Control is represented as both PRE and POST for simplicity of comparisons across treatments. PRE treatments not sharing a lowercase letter are significantly ($p<0.05$) different. POST treatments not sharing an uppercase letter are significantly different. All treatments were analyzed by ANOVA. Significant differences were analyzed by Tukey's HSD post-hoc test except POST-adductor which was analyzed by Fisher's LSD post-hoc test.

It is known that HPP can oxidize carotenoid pigments in salmon, evidenced by significantly lower a values compared to a raw control (Ojagh and others 2011). However, the results of this study demonstrate increases in both a and b values with pressure (Table 2.4). While increasing a values have not been reported in HPP fish or oysters, they have been reported in HPP chicken processed at 400 MPa for a single cycle (Del Olmo and others 2010). Abalone meat processed at 550 MPa for 8 min was reported to have L, a, and b values that did not significantly differ from unprocessed controls (Briones-Labarca and others 2012), which is in contrast to the values obtained in the current study. While pre-rigor processed foot a values were not significantly different from the control, post-rigor processed foot had significantly increasing a values with pressure, from 12.0 (control) to 14.1 (POST-5-300). For adductor, both pre-rigor and post-rigor processed a values increased with pressure, from 2.7 (control) to 5.3 (PRE-5-300) and 4.4 (POST-5-300). Despite increasing with pressure, a values for post-rigor processed samples for both foot and adductor had significantly lower a values than pre-rigor processed samples.

Table 2.4. Hunter a values.

	Foot		Adductor	
	Pre-rigor	**Post-rigor**	**Pre-rigor**	**Post-rigor**
Control	12.0 ± 2.6 a	12.0 ± 2.6 b	2.7 ± 1.2 c	2.7 ± 1.2 bc
1-100	13.6 ± 2.3 a	11.7 ± 1.9 b	4.4 ± 1.7 abc	2.7 ± 1.0 bc
3-100	14.2 ± 1.9 a	11.6 ± 1.5 b	4.2 ± 1.1 abc	2.8 ± 1.0 bc
5-100	14.9 ± 2.7 a	12.2 ± 2.2 b	3.9 ± 1.2 bc	2.5 ± 1.6 c
1-300	15.1 ± 3.2 a	14.2 ± 1.3 a	5.9 ± 1.7 a	3.7 ± 0.9 abc
3-300	14.9 ± 2.6 a	14.1 ± 2.4 a	5.3 ± 1.1 ab	4.1 ± 0.9 ab
5-300	15.0 ± 2.4 a	14.1 ± 2.7 a	5.3 ± 1.9 ab	4.4 ± 1.3 a

Each value is the mean ± standard deviation ($n \geq 8$). Control is represented as both PRE and POST for simplicity of comparisons across treatments. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. All treatments were analyzed by ANOVA. Significant differences were analyzed by Tukey's HSD post-hoc test except post-rigor foot which was analyzed by Fisher's LSD post-hoc test.

The b values (Table 2.5) significantly increased with pressure irrespective of muscle type or rigor status. There were no significant differences in b values between pre-rigor processed and post-rigor processed foot samples, however pre-rigor processed adductor samples had significantly higher b values than post-rigor processed adductor regardless of treatment.

Table 2.5. Hunter b values.

	Foot		Adductor	
	Pre-rigor	**Post-rigor**	**Pre-rigor**	**Post-rigor**
Control	23.0 ± 1.8 bc	23.0 ± 1.8 b	10.8 ± 1.4 d	10.8 ± 1.4 d
1-100	22.6 ± 1.8 c	23.7 ± 1.6 b	14.5 ± 2.7 b	11.1 ± 1.5 d
3-100	22.7 ± 2.8 c	23.3 ± 1.0 b	15.2 ± 1.1 b	11.9 ± 1.8 bcd
5-100	25.2 ± 1.5 b	23.9 ± 1.8 b	15.3 ± 2.3 b	11.8 ± 2.1 cd
1-300	26.3 ± 1.8 a	26.8 ± 1.3 a	18.8 ± 1.5 a	13.4 ± 1.4 abc
3-300	26.1 ± 2.1 a	26.9 ± 1.4 a	16.5 ± 1.8 ab	14.7 ± 1.8 a
5-300	26.4 ± 1.7 a	26.3 ± 2.0 a	16.6 ± 1.7 ab	14.0 ± 1.9 ab

Each value is the mean ± standard deviation ($n \geq 8$). Control is represented as both PRE and POST for simplicity of comparisons across treatments. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different, analyzed by ANOVA (Tukey's HSD post-hoc).

There was no significant effect of processing time on the Hunter L, a, or b values for muscle type or rigor status. The adductor had significantly higher L values and lower a and b values than the foot as expected. Finally, post-rigor processed foot was significantly lighter, with higher L and lower a and b values, than pre-rigor processed foot irrespective of treatment. Considering the importance of light coloration to abalone consumers, post-rigor HPP of abalones may be a way to improve acceptance of highly pigmented abalone foot.

2.3.5. Texture Profile Analysis

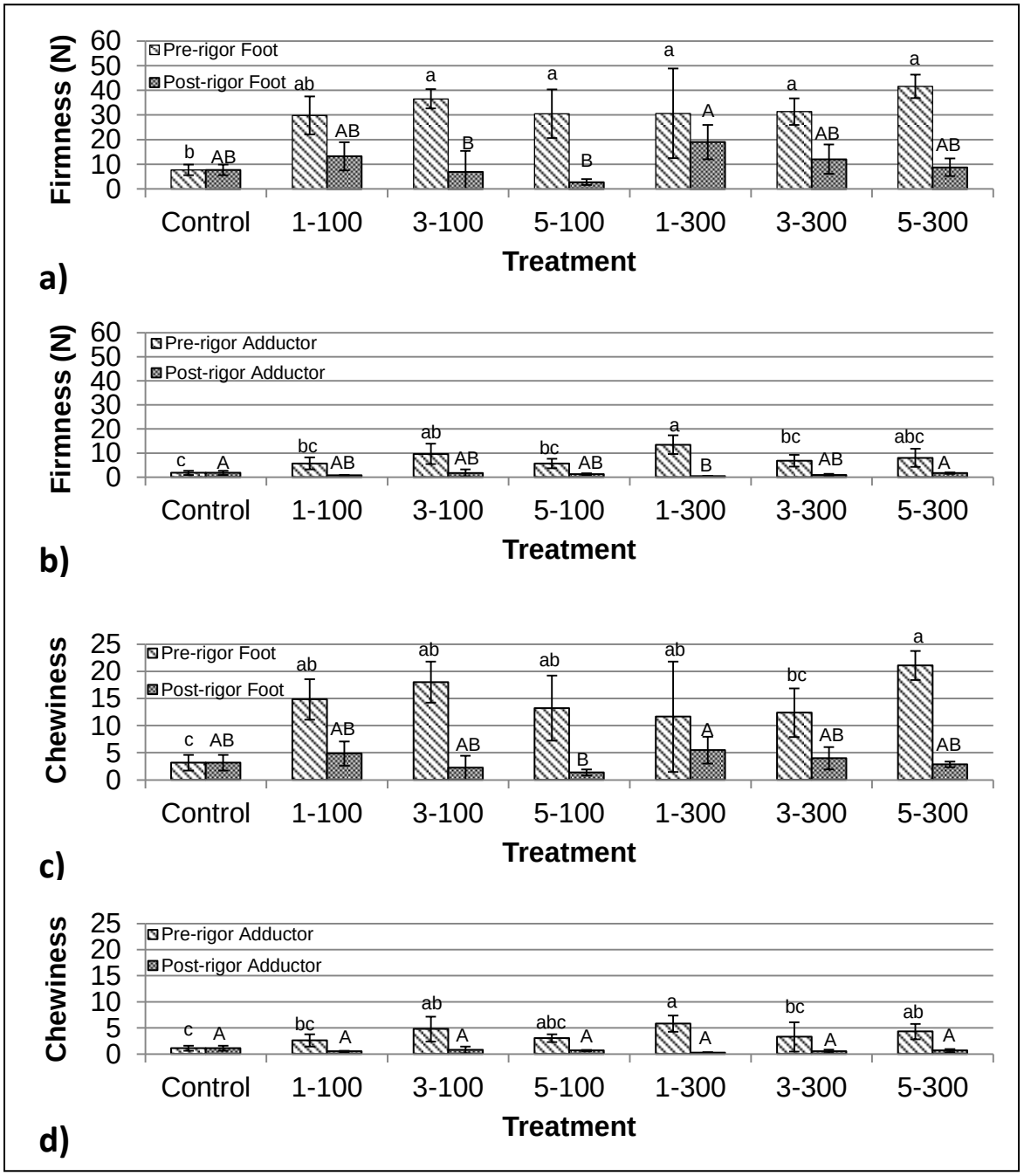
Pre-rigor processed meat significantly ($p<0.05$) toughened due to processing compared to both the control and the post-rigor processed treatments, which were not significantly different in firmness from the control (Figure 2.3). The post-rigor results are similar to those reported for abalones processed at 550 MPa for 5 min which were not significantly firmer than unprocessed controls (Briones-Labarca and others 2010). In this study, firmness was not significantly different between the control (7.7 N) and the POST-5-300 treatment (8.8 N), however firmness significantly increased for pre-rigor processed treatments (41.6 N for PRE-5-300). Results for adductor were similar, with firmness not being significantly different between the control (1.8 N) and POST-5-300 (1.7 N), but significantly increasing when processed pre-rigor (8.1 N for PRE-5-300). Overall, adductor meat was significantly ($p<0.05$) less firm (~5 times) and less chewy (~5-12 times) than foot meat.

The firm, crisp texture of raw abalone meat is highly desired, so preservation of the raw textural qualities of unprocessed meat is optimal (Olaechea and others 1993). Processing of pre-rigor meat caused substantial increases in firmness compared to the control and post-rigor processed meat, however, since firmness values for the highest processing parameters (5,300) of post-rigor meat were not significantly different from the control, pressures up to 300 MPa for 5 min could be used to process post-rigor abalones without toughening foot or adductor meat.

Pre-rigor processed foot and adductor were significantly more chewy than post-rigor processed meat (Figure 2.3), increasing from 2.9 (POST-5-300) to 21.0 (PRE-5-

300) for the foot, and increasing from 0.7 (POST-5-300) to 4.3 (PRE-5-300) for adductor. There were no significant differences between the post-rigor processed meat and the control, though the foot was three times as chewy as the adductor. It is likely that the differences observed between pre-rigor processed abalone and post-rigor processed abalone for both firmness and chewiness has to do with when the muscle first contracted after shucking. In pre-rigor processed meat, the first post-mortem contraction is after pressurization which may cause irreversible changes to the connective tissue compared to post-rigor processed, and control, abalone meat which contracts post-mortem during rigor (Kennick and others 1980). These results align with those for firmness, and suggest that processing post-rigor abalones will not cause an increase in chewiness of raw abalones.

Figure 2.3. Firmness and chewiness TPA values. Foot firmness (a), adductor firmness (b), foot chewiness (c), and adductor chewiness (d).

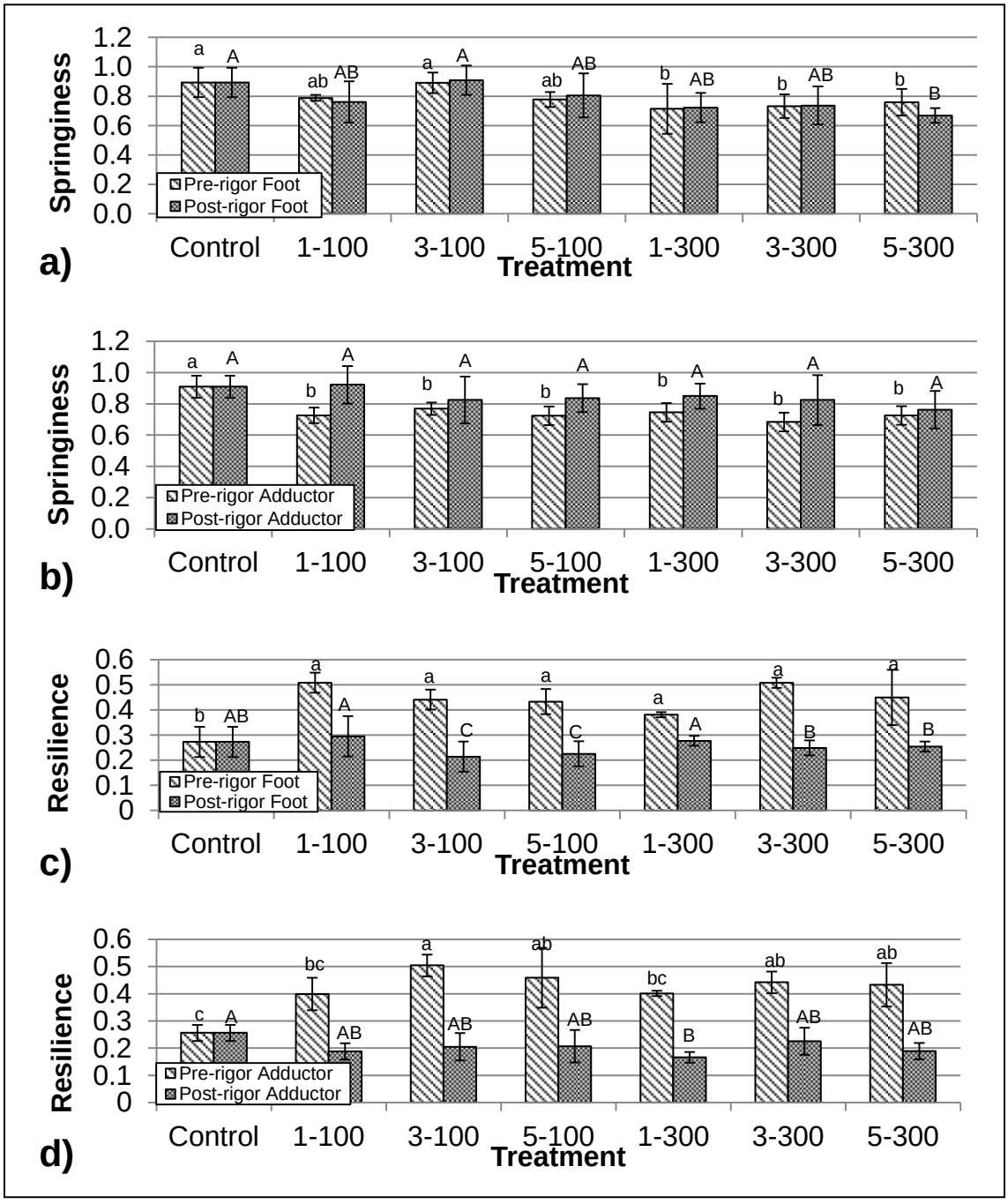


Each value is the mean ± standard deviation (n=6). Control is represented as both PRE and POST for simplicity of comparisons across treatments. PRE treatments not sharing a lowercase letter are significantly ($p<0.05$) different. POST treatments not sharing an uppercase letter are significantly different. All treatments were analyzed by ANOVA (Tukey's HSD post-hoc) except PRE-foot firmness, PRE-foot chewiness, and PRE-adductor chewiness which were analyzed by Kruskal-Wallis (Mann-Whitney post-hoc).

Springiness in the foot significantly decreased with HPP, regardless of rigor status, decreasing from 0.893 (control) to 0.759 (PRE-5-300) and 0.668 (POST-5-300) (Figure 2.4). Springiness also decreased with HPP for pre-rigor adductor from 0.909 (control) to 0.725 (PRE-5-300), but was not significantly different for post-rigor adductor, ranging from 0.909 (control) to 0.762 (POST-5-300). The decrease in springiness at the highest pressure and time combination for foot meat may suggest gelatinization of myofibrillar proteins, however, this trend was not seen in the post-rigor processed adductor treatments or in similar studies (Briones-Labarca and others 2012).

Pre-rigor processed foot samples were nearly twice as resilient as post-rigor processed samples, at 0.449 for PRE-5-300 compared to the significantly lower 0.254 for POST-5-300 (Figure 2.4). Adductor meat followed the same trend with values ranging from 0.433 for PRE-5-300 to 0.189 for POST-5-300. Conversely, post-rigor processed foot (0.254) and adductor (0.189) were not significantly different from controls (0.273 foot, 0.256 adductor), but pre-rigor processed foot (0.449) and adductor (0.433) had significantly greater resilience than controls (0.273 foot, 0.256 adductor). These results suggest that HPP up to 300 MPa for 5 min could be utilized to process post-rigor abalone without affecting the firmness, chewiness, or resilience compared to unprocessed raw abalone, despite springiness being negatively affected.

Figure 2.4. Springiness and resilience TPA values. Foot springiness (a), adductor springiness (b), foot resilience (c), and adductor resilience (d).



Each value is the mean ± standard deviation (n=6). Control is represented as both PRE and POST for simplicity of comparisons across treatments. PRE treatments not sharing a lowercase letter are significantly ($p<0.05$) different. POST treatments not sharing an uppercase letter are significantly different. All treatments were analyzed by ANOVA (Tukey's HSD post-hoc) except PRE-foot springiness, PRE-foot resilience, and PRE-adductor resilience which did not satisfy normality or homogeneity requirements and were analyzed by Kruskal-Wallis (Mann-Whitney post-hoc).

2.3.6. Shear Force

Shear represents cutting force, and together with TPA, gives a more comprehensive understanding of abalone textural attributes. Unlike the TPA results, the shear values did not demonstrate significant differences that followed specific trends related to pressure or rigor status, except for pre-rigor foot, but like TPA, foot meat required significantly greater shear force than adductor (Table 2.6). Pre-rigor foot shear values (PRE-5-300 was 33.8) were the only values significantly different from control values (39.8). It has been suggested that if post-mortem muscle contraction occurs due to the pressure treatment before onset of rigor, then connective tissue such as collagen may be disrupted enough to cause a tenderization effect (Kennick and others 1980). In this study, pre-rigor processed abalone meat would have experienced just such a contraction after processing, while post-rigor meat and the control would not have, possibly leading to the decrease in shear force reported for pre-rigor processed foot.

In contrast, post-rigor foot (34.6 POST-5-300) and pre- and post- adductor shear values (23.8 PRE-5-300, 28.6 POST-5-300) were not significantly different from respective controls (39.8 foot, 24.3 adductor). Foot samples had significantly higher shear values than adductor samples, as expected based on TPA firmness. TPA and shear results shared similar values for pre-rigor foot samples, 41.6 N for firmness (PRE-5-300) and 33.8 N for shear (PRE-5-300). Post-rigor foot samples, however, were significantly firmer when evaluated by shear (34.6 N POST-5-300) than by TPA (8.8 N POST-5-300). While HPP up to 500 MPa was shown to reduce shear force values in beef from 5.9 N (unprocessed control) to 4.3 N (100 MPa) and 4.9 N (500 MPa) (Ichinoseki and others 2006), the higher shear force values observed in this study were likely due to the much

higher collagen content in abalone (3.5%) compared to beef (0.29-0.57%) (Nakamura and others 2010). Collagen has been shown to only be minimally affected by HPP (Ichinoseki and others 2006) so the lack of difference in shear values among treatments is not unexpected.

Table 2.6. Shear force (N) values.

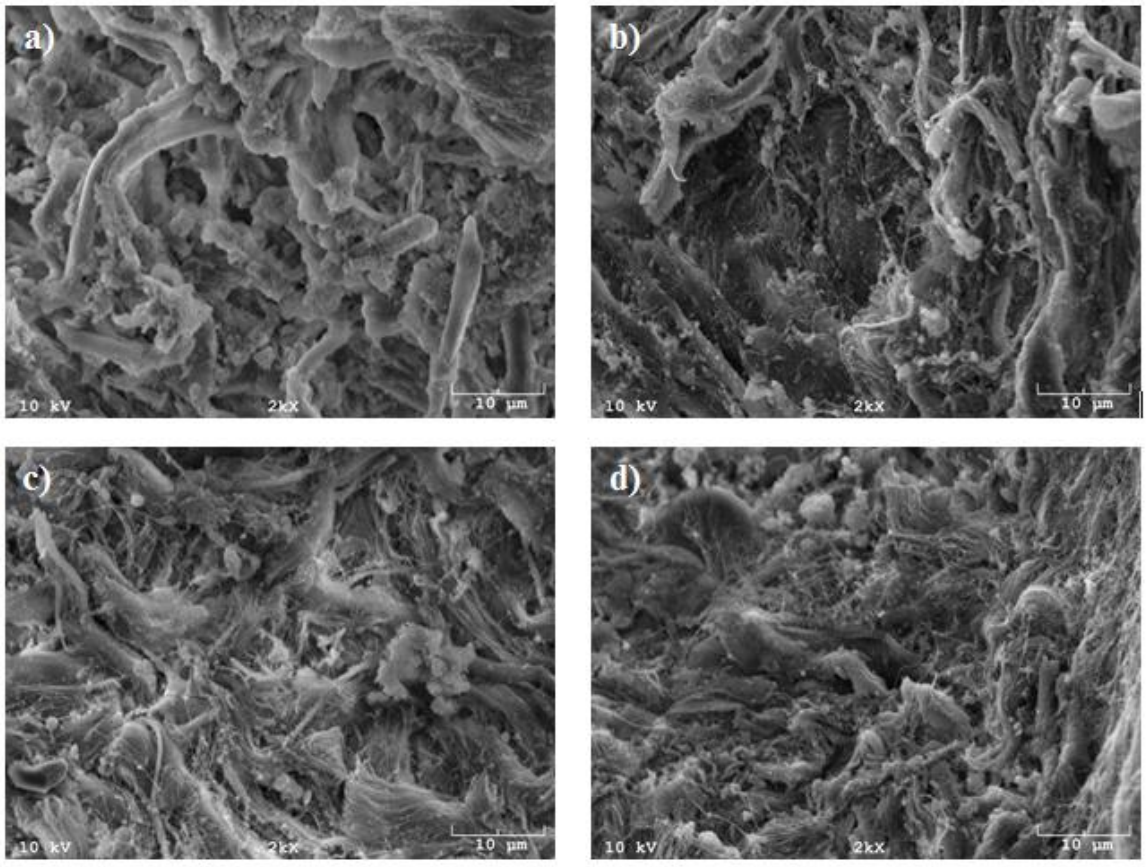
	Foot		Adductor	
	Pre-rigor	**Post-rigor**	**Pre-rigor**	**Post-rigor**
Control	39.8 ± 5.6 a	39.8 ± 5.6 a	24.3 ± 3.8 a	24.3 ± 3.8 a
1-100	35.0 ± 1.9 ab	28.6 ± 3.9 c	21.2 ± 5.9 a	28.9 ± 5.2 a
3-100	33.7 ± 2.7 b	38.8 ± 7.4 ab	26.5 ± 3.7 a	26.7 ± 4.4 a
5-100	36.1 ± 3.2 ab	29.5 ± 6.9 bc	29.4 ± 4.4 a	26.9 ± 7.3 a
1-300	32.5 ± 1.4 b	37.0 ± 2.5 abc	26.6 ± 5.9 a	27.1 ± 4.5 a
3-300	35.4 ± 2.3 ab	39.7 ± 2.7 a	23.1 ± 5.8 a	32.3 ± 4.6 a
5-300	33.8 ± 3.1 b	34.6 ± 1.8 abc	23.8 ± 4.9 a	28.6 ± 4.2 a

Each value is the mean ± standard deviation (n=6). Control is represented as both PRE and POST for simplicity of comparisons across treatments. Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. All treatments were analyzed by ANOVA (Tukey's HSD post-hoc).

2.3.7. Scanning Electron Microscopy

Adductor SEM micrographs showed clear processing effects when comparing the control to HPP samples (Figure 2.5). At higher magnification (2000x), small fibrils (<1 μm) are observed in all adductor images except the control. The presence of these fibrils suggests pressure-induced unraveling of collagen fibers making up the endomysium, which sheaths each myofibril. It has been shown in beef that HPP can cause stretching and changing of the endomysium, though changes in texture are minimized due to the continued presence of collagen in the tissue (Ichinoseki and others 2006).

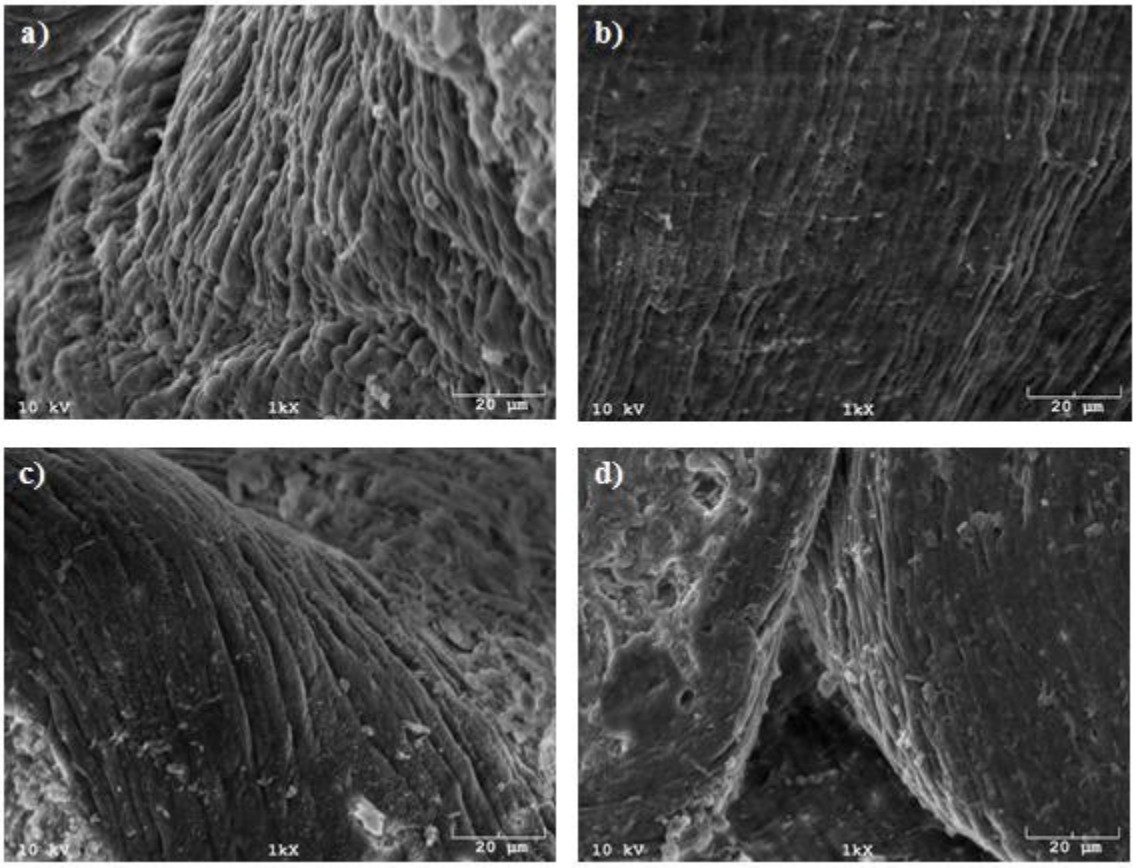
Figure 2.5. Scanning electron micrographs of abalone adductor meat. Control (a), PRE-1-100 (b), PRE-5-300 (c), POST-5-300 (d) taken at 2000x. Scale bars represent 10 µm.



Foot muscle images (Figure 2.6) show little difference among processing treatments, except for PRE-1-100 which had significantly ($p<0.05$) more narrow myofibrils (2.0 µm) than the control (2.7 µm). A significant difference between pre-rigor processed and post-rigor processed samples was not observed at the highest processing parameters tested, with myofibril widths ranging from 3.0 µm (PRE-5-300) to 2.5 µm (POST-5-300). The lack of visible difference in myofibril width between pre-rigor and post-rigor processed foot corresponds with the lack of difference observed for shear values. The endomysium sheath may have prevented detection of myofibril changes

despite being weakened by HPP, observed in the current study as well as in HPP beef (Ichinoseki and others 2006).

Figure 2.6. Scanning electron micrographs of abalone foot meat. Control (a), PRE-1-100 (b), PRE-5-300 (c), POST-5-300 (d) taken at 1000x. Scale bars represent 20 µm.



The lack of difference between the control (2.7 µm) and POST-5-300 (2.5 µm) in the foot images is consistent with texture results. There was also no significant difference in foot images between PRE-5-300 (3.0 µm) and the control (2.7 µm) which was unexpected since the TPA value for PRE-5-300 firmness was six times higher than control firmness. The lack of significant difference observed in foot SEM images does support shear data, however, pointing again to collagen interference. Exploration of the

collagen matrix by SEM may further corroborate texture analyses as well as differences observed between foot and adductor muscles.

2.3.8. Shucking Observational Study

Abalones processed for 5 min at 300 MPa were easily removed from their shells using only gentle pressure from fingers. By contrast, unprocessed abalones required substantial force and the use of a spoon to remove the meat from the shell. Further investigation using a qualitative scale to assess different pressure and time combination on shucking ease is recommended for future studies, but initial observation suggests HPP could reduce shucking time and meat damage compared to traditional hand shucking.

2.3.9. Microbiological Observational Study

The minimum HPP parameters of 1 min at 100 MPa resulted in a 1.5 log APC reduction from unprocessed control counts. The control had 2.8×10^5 CFU/g while the 1-100 processed abalone had 1.4×10^4 CFU/g. A similar log reduction in APC bacteria was reported in abalones processed for 8 min at 550 MPa (Briones and others 2010) so a study of abalone shelf-life following HPP at pressures below 500 MPa is merited since lower processing pressures are associated with higher quality texture in beef.

2.4. Conclusions

HPP at 300 MPa for 5 min caused significant toughening of pre-rigor abalone meat as evidenced by increased firmness, chewiness, and resilience values. Compared to pre-rigor processed abalones, post-rigor processed abalones were lighter in color and more tender, suggesting that waiting for rigor to resolve prior to processing abalones

would be beneficial for product quality attributes. Notably, the texture of post-rigor processed abalone was not significantly affected by HPP at 300 MPa for 5 min suggesting that holding abalone meat through rigor resolution will produce raw meat of equal texture attributes to unprocessed meat. Additionally, post-rigor processed abalone foot meat increased in lightness which is desirable to abalone consumers and could increase market value of highly pigmented abalones.

Processing of live in-shell abalone at 300 MPa for 5 min was successful in reducing the force required to shuck the meat from the shell, which could provide labor savings for the abalone industry. Additionally, a 1.5 log reduction of aerobic bacteria was achieved in shucked abalone processed at 100 MPa for 1 min which suggests that abalone shelf-life could be extended with even mild HPP treatment. Further investigation into the role of collagen in preventing textural changes of abalone meat during HPP is warranted, as well as exploration of the shelf-life of abalone processed at lower processing pressures.

CHAPTER 3

EFFECT OF BOILING ON POST-RIGOR HIGH PRESSURE PROCESSED FARM-RAISED ABALONE (*Haliotis rufescens*) QUALITY

3.1. Objectives

High pressure processing (HPP) has been explored for raw abalone meat at pressures of 500 and 550 MPa without affecting texture (Briones-Labarca and others 2012), but its effects on subsequently cooked HPP abalone qualities have not been explored. Abalone may be consumed raw, but is commonly cooked for 3 or more hours to tenderize the collagen-rich meat (Gao and others 2001). Abalone texture is described as firm and crisp when raw, and soft and chewy when cooked (Brown and others 2008). The transformation in texture has been attributed to the high collagen content of abalone, as much as 4 times as high as beef, gelatinizing due to heat treatment (Olaechea and others 1993). Collagen may contribute to the lack of textural difference between HPP abalone meat and unprocessed abalone meat due to the structural integrity of collagen fibers (Hatae and others 1995, Sanchez-Brambila and others 2002a, Briones-Labarca and others 2012). Collagen has been shown in beef to have increased surface area and changes in structure, described as stretching, following HPP suggesting the potential for changes in HPP abalone texture once cooked (Ichinoseki and others 2006).

HPP has been shown to be effective for shucking bivalves such as oysters (He and others 2002), but its effects on shucking of single shelled abalone have not been evaluated. The labor savings of HPP shucking could have enormous benefit for the abalone industry which currently relies exclusively on hand shucking. The objectives of

this study were to assess 1) the effect of boiling on HPP abalone texture and color, 2) effects of different HPP parameters (pressure and time) on texture and color of raw and cooked abalone meat, and 3) effects of HPP on shucking of abalones.

3.2. Materials and Methods

The study had a 2x2x2 multifactorial design (Table 3.1). Abalones were processed at either of two pressures (100 or 300 MPa) for two times (5 or 10 min), and then either left raw or boiled. Unprocessed abalones were used as controls for both raw and cooked samples. Unprocessed, whole, raw abalones were used to evaluate moisture, protein, and collagen contents. Subsamples from the foot muscle of each treatment were used to evaluate water holding capacity, texture, color, and ultrastructure. A total of 133 abalones were used for this study. There were 12 abalones for each of the ten treatments (no replicates), eight raw unprocessed abalones for collagen analyses, and five live unshucked abalones for evaluation of the effect of HPP on shucking. Reagents were analytical grade and were purchased from Fisher Scientific (Waltham, MA) unless otherwise noted. Texture, color, and moisture analyses were conducted within four days of processing. Scanning electron microscopy (SEM) and protein samples were prepared within 12 days of processing. Collagen analyses began within 19 days of processing. Values are presented on a wet-weight basis (wwb).

Table 3.1. Experimental design.

Processing Parameters	Raw	Cooked
Pressure (MPa)	100, 300	100, 300
Time (min)	5, 10	5,10

3.2.1. Processing

Live, July-harvested, farm-raised abalones (*Haliotis rufescens*) (n=133) (The Abalone Farm, Cayucas, CA) were divided into ten treatments according to the experimental design. The in-shell abalone (n=117) mean weight was 80.2 g ± 10.2 and the mean shell length was 82.7 mm ± 6.7. Abalones had not been fed for a minimum of 5 days prior to shucking. Abalones were shucked, eviscerated, and scrubbed to remove the epipodium pigment. Shucked meats were loosely packed 20 per bag (Winpak, Films Inc., Senoia, GA) and stored at 1°C 16-21 h to allow mucus production to cease. Following that time period, the abalones were rinsed and packed 6 to a bag at 99% vacuum (Model UV550, Koch Industries, Wichita, KS). Bags were kept on ice during transport to the Natick Soldier Research, Development, and Engineering Center (Natick, MA). Controls were kept in ice in coolers in the Matthew Highlands Pilot Plant (Orono, ME). A 1 L HPP unit (Engineering Pressure Systems Inc., Haverhill, MA) was used and processing was randomized among treatments. The temperature of the vessel during pressurization ranged from 20°C-25°C. Hydraulic fluid (20:1 water:Hydrolubric 120-B (Houghton International Inc., Norristown, PA)) was used to achieve hydrostatic pressure. The come-up time ranged from 3 min to 4.5 min and depressurization was immediate.

3.2.2. Moisture and Protein Content

Moisture content was determined gravimetrically by drying at least duplicate 3 g unprocessed, chopped abalone samples for 24 h in a 105°C oven (Fisher Isotemp, Barrington, IL) (AOAC 2005). Protein content was determined in triplicate from pre-dried samples with a combustion nitrogen analyzer (Rapid N III, Elementar Americas

Inc, Mount Laurel, NJ). Aspartic acid (Sigma-Aldrich, St. Louis, MO) was used as the nitrogen standard, and a conversion factor of 6.25 was used to determine crude protein content. Both moisture and protein contents are reported as g/100 g (wwb).

3.2.3. Collagen Content

Collagen may be qualified as the total amount of collagen in g/100 g meat, or by its solubility in acid or pepsin. Total collagen was evaluated following the AOAC method (AOAC 1995) for hydroxyproline in meat. Raw unprocessed whole abalones (n=8) were chopped and 4 g from each were added to 30 mL 7 N H_2SO_4. Gelatin (Knox, Deerfield, IL) was used as a control and 0.5 g was added to 30 mL H_2SO_4 for hydrolysis. Acidified samples were placed in a 105°C oven (Fisher Isotemp, Barrington, IL) for 16 h, cooled, and quantitatively brought to 500 mL with deionized (DI) water. Solutions were filtered and stored at 4°C for a maximum of 2 weeks. Final dilutions of the solutions were obtained by bringing 2 mL to 100 mL with distilled water, and then using 2 mL of that concentration directly for spectrophotometric quantification.

Acid and pepsin soluble collagen fractions were extracted following established methods (Sato and others 1988, Espe and others 2004). For the alkali pre-extraction, 2.5 g abalone was microhomogenized for 30 s (Polytron, Brinkmann, Westbury, NY) in 10 volumes 0.1 M NaOH. The mixture was centrifuged at 10,000 *g* for 20 min (model J2-21, Beckman Coulter, Brea, CA) and the supernatant discarded. To the pellet, 20 volumes 0.1 M NaOH were added and the solution was vortexed before being placed on a shake plate (model 1314, Labline Instruments, Melrose Park, IL) at 4°C for 12 hours. The latter alkali extraction was repeated 3 more times. The final pellet was washed by

immersing it in DI water, vortexing, and re-centrifuging at 10,000 *g* for 20 min prior to acid soluble collagen (ASC) extraction.

The water-washed alkali pellets were vortexed with 10 volumes 0.5 M acetic acid and left on a shake plate at 4°C for 2 days. The solution was centrifuged at 10,000 x *g* for 20 min and the supernatant volume was quantified and retained as the ASC fraction. For the pepsin soluble collagen (PSC) extraction, the acid pellet was vortexed with a 20:1 ratio of original sample mass:pepsin (porcine, 400 units/mg, Sigma-Aldrich, St. Louis, MO) and 10 volumes 0.5 M acetic acid, then placed on a shake plate at 4°C for 2 days. The solution was centrifuged at 10,000 *g* for 20 min and the supernatant volume was quantified and retained as the PSC fraction. The pellet was retained as the insoluble collagen (ISC) fraction.

Hydrolysis of the soluble collagen fractions was completed by adding 1 mL 60% H_2SO_4 to 1 mL of each soluble collagen fraction and the whole ISC pellet then placing them in a 105°C oven for 12 h. Hydrolyzed samples were neutralized to pH 6-7 with 5 N NaOH. The ASC fraction was brought to 10 mL with DI water, and 2 mL were used directly for spectrophotometric quantification. The PSC and ISC fractions were brought to 100 mL with DI water, and 2 mL were used directly for quantification. The hydroxyproline (Sigma-Aldrich, St. Louis, MO) standard curve was made fresh daily. The chloramine-T (Sigma-Aldrich, St. Louis, MO) oxidant solution, 4-dimethylaminobenzaldehyde (Sigma-Aldrich, St. Louis, MO) color reagent, and acetate-citrate buffer were made as described in the AOAC method (AOAC 1995). To each 2 mL aliquot of final dilution was added 1 mL oxidant solution, vortexed, and allowed to stand at room temperature for 20 min. The color reagent, 1 mL, was then added,

vortexed, and immediately immersed in a water bath maintained at 60°C for exactly 15 min, cooled, and absorbance was read in a spectrophotometer (DU530, Beckman Coulter, Brea, CA) at 558 nm. Hydroxyproline concentration (g/100 g) was determined by comparing sample absorbance to the hydroxyproline standard curve. The collagen content (g/100 g) was calculated using a conversion factor of 9.8 since abalone collagen is reported to contain 10.2% hydroxyproline (Kimura and Kubota 1968).

3.2.4. Colorimetric and Texture Analyses

Cooked treatments were boiled the same day as texture and color analyses. Whole abalones were sliced horizontally below the base of the adductor to separate the foot from the adductor muscle. A digital caliper was used to measure 20x20x10 mm square plugs which were cut, one per abalone, from the center of each foot muscle on the ventral side. Plugs for raw treatments were stored in 7.5 x 12.5 cm WhirlPak (Nasco, Fort Atkinson, WI) bags at 4°C while plugs for cooked treatments were placed in a single layer in a metal strainer and immersed in boiling water (99.3°C-99.5°C) for 15 s. The time was determined based on preliminary evaluations of textural changes of cooked non-HPP abalones, with undesirable chewiness increasing with time at and above 20 s. Internal temperature of the center of one plug per treatment was evaluated with a thermocouple. Temperatures ranged from 41°C-66°C. Following temperature reading, the cooked plugs were immediately placed on a metal sheet pan in a single layer and placed in the refrigerator to maximize cooling rate.

3.2.4.1. Color

Colorimetric analyses were performed on the abalone muscle plugs (n=12) using a colorimeter (LabScan XE, Hunter Labs, Reston, VA). The Hunter L, a, b values were recorded by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs, Reston, VA). Whiteness was calculated by the equation $W=100-((100-L)^2+a^2+b^2)^{0.5}$. The colorimeter was standardized using white and black tiles for a port size of 25.4 mm and an area view of 30.5 mm. After the initial color reading, each plug was rotated 120º. The rotation was repeated a final time to achieve three readings, which were then averaged to one value per plug.

3.2.4.2. Texture Profile Analysis

Following color analysis, individual foot muscle plugs (n=12) were placed on the calibrated texture analyzer platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) ventral side up. The texture analyzer was configured with a 10 mm cylindrical probe, 5 mm/s test speed, two 30% (3 mm) compressions, and a 5 s gap between compressions. TPA emulates chewing, and can be useful for comparison to the consumer experience. Force (N), area (N*s), distance (mm) between peak heights, and time (s) were recorded by the texture analysis software (Exponent 32, version 5,0,6,0 2010, Texture Technologies Inc., Scarsdale, NY) to calculate TPA parameters.

The five parameters evaluated were firmness, springiness, cohesiveness, chewiness, and resilience. Firmness is resistance to compression, determined by the peak force (N) of the first compression (Bourne 2002). Springiness represents how well a product springs back after compression, calculated by dividing the distance to peak force

of the second compression by the distance to the peak force of the first compression (Bourne 2002). Cohesiveness corresponds to how a product resists second compression compared to the first, a unitless number calculated by dividing the work area of the first compression by the work area of the second compression (Bourne 2002). Chewiness characterizes elastic resistance of a solid food, a unitless number calculated by multiplying hardness, cohesiveness, and springiness (Bourne 2002). Resilience describes the immediate springiness as the probe is withdrawn between "bites", a unitless number calculated by dividing the area of the withdrawal of the first compression by the area of the first compression (Bourne 2002).

3.2.5. Water Holding Capacity and Moisture Content

Duplicate abalone foot plugs from each treatment were weighed, wrapped in 4 pieces of pre-weighed Whatman #1 filter paper, placed in 50 mL falcon tubes, and centrifuged at 1000 x *g* for 15 minutes in a bench top centrifuge (model 5430, Eppendorf, Hamburg, Germany). Following centrifugation, filter paper was re-weighed and the difference was recorded. Water holding capacity (WHC) was calculated as the percent of water retained by the meat with respect to water present in meat prior to centrifugation. Moisture contents of foot plugs were determined gravimetrically by drying duplicate 5 g chopped abalone overnight in a 105°C oven (Fisher Isotemp, Barrington, IL), and are reported as g/100 g (AOAC 2005).

3.2.6. Scanning Electron Microscopy

Raw and cooked abalone samples were prepared for microwave enhanced fixation by using a razor blade to slice pieces (n=3) no thicker than 1 mm from within 3 mm of

the edge of the foot muscle plug of each treatment. Stock 0.2 M phosphate buffer was made by combining 0.22 g $NaH_2PO_4 \cdot H_2O$, 1.19 g Na_2HPO_4, and 8 g sucrose in 50 mL deionized water to a final pH of 7.3. Working buffer was made by making a 1:1 ratio of stock phosphate buffer:deionized water to a final concentration of 0.1 M, with the addition of 1 drop 1% $CaCl_2$ per 10 mL buffer. Specimens were fixed in 2.5% glutaraldehyde (Electron Microscopy Services, Hatfield, PA), made by combining 3 mL 25% glutaraldehyde, 12.5 mL stock buffer, and 2 drops $CaCl_2$. The solution of 1% osmium tetroxide (OsO_4) (Electron Microscopy Services, Hatfield, PA) was prepared by combining 2% OsO_4 in equal parts stock buffer.

Iced specimens, in vials, were fixed in approximately 2 mL 2.5% glutaraldehyde solution in a pre-warmed microwave oven for 1 7 s cycle on high, followed by a 20 s rest, and a final 7 s cycle on high (7/20/7). The initial fixation was followed by a replacement of fixative with working buffer and a 7/20/7 cycle. The buffer rinse was repeated once for a total of two rinses. The rinsed specimen was then fixed with 1% OsO_4 for 1 7/20/7 cycle and rinsed with deionized water. Fixed specimens were dehydrated in serially increasing concentrations of ethanol (50%, 70%, 95%) for two 7/20/7 cycles at each level. Following the 95% dehydration, samples were further dehydrated in 100% ethanol three times for 7 min each time at room temperature. The samples were stored in 100% ethanol until critical point drying.

Critical point drying (Samdri PVT-3, Tousimis Research Corp., Rockville, MD) was achieved in a pre-cooled specimen chamber in 100% ethanol which was gradually replaced with liquid carbon dioxide in five 5 min soaks. After the last soak, the chamber was 75% filled with liquid carbon dioxide and the heater was turned on until the chamber

reached 45°C and pressure no greater than 1400 psi. The chamber was very slowly exhausted and the dried samples were transferred to stubs affixed with carbon-coated tape and silver adhesive (503, Electron Microscopy Sciences, Hatfield, PA). The samples were sputter coated (Cressington 108 Auto, Redding, CA) at 40 mA and 0.08 mbar for 90 s to generate a 35 nm layer of gold-palladium on the surface. Samples were stored in a dessicator until imaging. The scanning electron microscope (AMRay 1820 Digital SEM, Bedford, MA) was degaussed initially and between samples. An accelerating potential of 10 kV and a spotsize of 10 were selected, and magnification up to 1,000 times (1000x) was used.

3.2.7. Shucking Evaluation

Live unshucked vacuum packaged abalones (n=5) were processed individually at each parameter (5 or 10 min at 100 or 300 MPa) with one reserved for an unprocessed control. Abalones were stored at 4°C for six days after processing, then evaluated by the author for ease of removal from the shell using a qualitative 1-5 scale (Table 3.2).

Table 3.2. Shucking evaluation scale.

Scale	Description
1	No force exerted; slipped out of shell.
2	Slight force exerted.
3	Medium force exerted.
4	High force exerted.
5	Extremely high force exerted; required a utensil.

3.2.8. Statistical Analyses

Data were analyzed using SYSTAT 12 (Systat Software, Chicago, IL) for one-way analysis of variance (ANOVA) for all one-level (treatment) analyses, and multi-way ANOVA to assess multiple independent variables. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses. The Shapiro-Wilk test for normality and the Levene equality of variances (homogeneity) test were assessed prior to running ANOVA. In cases where data did not satisfy either normality or homogeneity, they were evaluated non-parametrically using Kruskal-Wallis. Mann-Whitney was selected for non-parametric post-hoc analyses. For all statistics, a significance level of $p<0.05$ was selected.

3.3. Results and Discussion

3.3.1. Moisture, Protein, and Collagen Content

The moisture and protein contents of the whole abalone meat were 77.8 g/100 g and 18.1 g/100 g respectively (Table 3.3). These values are similar to those found in the previous chapter which reported moisture content of 75% and protein content of 18%. Similarly, values reported for whole farmed red abalone were reported to be 73.9% moisture and 21.6% protein (Briones-Labarca 2012). The total collagen content of these abalones was slightly lower than those in chapter 2, at 2.990 g/100 g meat compared to 3.545 g/100 g meat in the previous study. The abalones harvested for the previous study were on average 8 g larger than those harvested for this study, which may explain the decrease in collagen despite a controlled diet, environment, and harvest season.

Regarding collagen solubility, however, there was a drastic change from the previous year to this year. Acid soluble collagen (ASC) increased from 0.007-0.013 g/100 g meat to 0.05 g/100 g meat, pepsin soluble collagen (PSC) was nearly constant at 0.794-0.772 g/100 g meat to 0.737 g/100 g meat, and insoluble collagen (ISC) dropped from 1.008-1.163 g/100 g meat to 0.071 g/100 g meat. There are no other published reports of these types of soluble collagen in abalone to compare, but it is possible that the significant changes from season to season were due to small differences in the animal muscle as well as the susceptibility of the collagen extraction methods to large standard deviations.

Table 3.3. Moisture, protein, and collagen content of whole abalone (g/100 g meat).

	Whole Abalone
Moisture	77.818 ± 0.736
Protein	18.141 ± 0.684
Total Collagen	2.990 ± 0.225
Acid Soluble Collagen	0.050 ± 0.011
Pepsin Soluble Collagen	0.737 ± 0.042
Insoluble Collagen	0.071 ± 0.024

Each value is the mean ± standard deviation (n=3 for moisture and protein, n=8 for collagen).

3.3.2. Water Holding Capacity and Moisture Content of Foot

Water holding capacity (WHC) of abalone meat averaged 82.3% for raw treatments and 85.1% for cooked treatments (Table 3.4). There were no significant ($p>0.05$) differences in WHC regardless of pressure, time, or cooked status which was contrary to expectations. WHC is a measure of functionality of proteins related to their ability to retain water, and as muscle proteins denature due to high pressure or other treatments, WHC is known to decrease (Fernández and others 2007, Souza and others

2011). There was a significant decrease in moisture content from the raw (77%) to the cooked (76%) samples which has been described in other abalone research (Gao and others 2001, Hatae and others 1996). It appeared in the cooked samples that WHC increased with pressure and time from 79.5% to 87.7%, but this observation was not statistically significant. WHC was not conducted immediately following processing, so it is possible that differences were obscured by proteolytic activity between processing and the WHC evaluation.

Table 3.4. Water holding capacity (%) of whole abalone.

	Raw	**Cooked**
Control	80.07 ± 5.50 aA	79.47 ± 0.77 aA
5-100	86.71 ± 3.72 aA	83.86 ± 6.10 aA
10-100	85.43 ± 2.43 aA	86.86 ± 1.14 aA
5-300	76.63 ± 4.31 aA	87.38 ± 4.04 aA
10-300	82.73 ± 0.71 aA	87.71 ± 0.02 aA

Each value is the mean ± standard deviation (n=5). Values within columns not sharing a lowercase letter are significantly ($p<0.05$) different. Values within rows not sharing an uppercase letter are significantly ($p<0.05$) different. Treatments were analyzed by ANOVA (Tukey's HSD).

3.3.3. Color

Cooked abalone meat had significantly ($p<0.05$) lower L and whiteness values than raw abalone meat (Table 3.5). Cooked meat significantly increased in L values with pressure, from 39.92 (control) to 42.74 (10-300) compared to raw meat which did not change significantly with pressure and ranged in L value from 45.63 (control) to 48.01 (10-300). The lack of significant difference among raw samples may be due to high standard deviations compared to those of the cooked samples obscuring differences due to pressure. Whiteness values increased significantly with pressure in cooked meat but

not in raw meat. The decrease in L and whiteness for cooked meat may be attributed to an increase in opacity which would reduce transmission of the colorimeter.

Values for a were not significantly different between raw and cooked treatments, but within raw treatments a values for HPP treatments were all significantly higher than for the control (Table 3.5), which was observed in the previous study (chapter 2) as well. Values for b similarly were not significantly different between raw and cooked treatments, except for 10-100 which had significantly higher b values when raw than when cooked (Table 3.5). Values for b did significantly increase with pressure for both raw and cooked, ranging from 15.09 (control) to 17.40 (10-300) for raw treatments and 16.30 (control) to 18.22 (10-300) for cooked treatments.

Hunter L, a, b values from this study were much lower than those discussed in chapter 2. L values ranged from 62.50 (control) to 73.80 (5-300) in chapter 2 compared to 45.63 (control) to (49.07 (5-300) in the current study. Values for a in chapter 2 ranged from 11.98 (control) to 14.14 (5-300) compared to those reported in the current study of 8.03 (control) to 9.80 (5-300). The values for b in chapter 2 ranged from 23.03 (control) to 26.29 (5-300) compared to 15.09 (control) to 17.61 (5-300) in the current study. The increase in pigmentation, represented by lower L values, may be due to changes in diet from year to year since the farmed abalone are fed kelp which may differ from year to year.

Similar abalone studies exploring effects of cooking did not report color data for comparison, but a review of the effect of cooking on meat color found that cooked meat color was dependent on a multitude of factors including type of meat, pH, duration of

heat treatment, and the sensitivity of muscle pigments to oxygen and heat (King and Whyte 2006). While cooking significantly decreased the L and whiteness values of abalone meat, the whitening effect due to HPP was evident.

Table 3.5. Hunter L, a, b, and whiteness values.

	L		a		b		Whiteness	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Control	45.63 ± 2.41 abA	39.92 ± 2.42 cB	8.03 ± 1.12 bA	8.31 ± 1.96 aA	15.09 ± 0.98 cA	16.30 ± 1.87 cA	42.98 ± 2.19 aA	37.13 ± 2.14 bB
5-100	45.84 ± 3.35 abA	40.45 ± 2.06 bcB	10.02 ± 0.85 aA	9.27 ± 1.35 aA	16.99 ± 1.10 abA	17.46 ± 0.96 abcA	42.32 ± 2.93 aA	37.24 ± 1.97 bB
10-100	44.28 ± 3.11 bA	40.38 ± 1.90 bcB	8.48 ± 1.48 abA	8.90 ± 1.22 aA	15.59 ± 1.14 bcB	16.75 ± 1.38 bcA	41.49 ± 3.00 bA	37.40 ± 1.68 abB
5-300	49.07 ± 3.98 aA	43.36 ± 2.57 aB	9.80 ± 1.86 aA	9.63 ± 1.94 aA	17.61 ± 1.70 aA	18.82 ± 2.07 aA	45.14 ± 3.49 aA	39.47 ± 2.06 aB
10-300	48.01 ± 2.35 aA	42.74 ± 1.93 abB	9.92 ± 1.55 aA	9.15 ± 1.23 aA	17.40 ± 1.11 aA	18.22 ± 1.20 abA	44.25 ± 2.18 aA	39.19 ± 1.73 abB

Values not sharing a lowercase letter are significantly different within columns. Values not sharing an uppercase letter are significantly different within rows for grouped columns (L, a, b, Whiteness).

3.3.4. Texture

There were no significant ($p>0.05$) differences in firmness, chewiness, springiness, or cohesiveness within raw and cooked treatments (Tables 3.6 and 3.7). The lack of difference among cooked samples irrespective of pressure or time suggests that HPP at up to 300 MPa for 10 min will not affect the texture of subsequently boiled abalone meat. Comparable studies are not available in the literature for cooked HPP abalone meat. While cooked HPP pork, beef, sheep, and ox meat have been evaluated for texture and found to be more tender than non-HPP cooked meat, differences were only reported between HPP and control treatments within raw and cooked samples and not in relation to pressure or time (MacFarlane 1973, Souza and others 2011).

Table 3.6. Firmness and chewiness TPA values of raw and cooked abalone.

	Firmness (N)		Chewiness	
	Raw	**Cooked**	**Raw**	**Cooked**
Control	3.914 ± 2.788 aA	6.677 ± 2.936 aA	2.223 ± 1.459 aB	4.861 ± 3.770 aA
5-100	3.608 ± 3.117 aA	8.192 ± 7.609 aA	1.593 ± 1.093 aB	5.977 ± 4.625 aA
10-100	6.302 ± 6.775 aA	8.856 ± 6.099 aA	2.397 ± 2.086 aB	6.209 ± 3.247 aA
5-300	4.538 ± 2.794 aB	10.337 ± 6.374 aA	2.208 ± 1.204 aB	8.340 ± 4.756 aA
10-300	7.852 ± 6.659 aA	10.323 ± 5.360 aA	3.697 ± 2.584 aB	8.378 ± 3.191 aA

Each value is the mean ± standard deviation (n=12). Values not sharing a lowercase letter are significantly different within columns, analyzed by ANOVA (Tukey's HSD post-hoc) for all treatments except raw firmness for which Kruskall-Wallis (Mann-Whitney post-hoc test) was used . Values not sharing an uppercase letter are significantly different within rows for grouped columns (Firmness, Chewiness), analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test).

Table 3.7. Springiness, cohesiveness, and resilience TPA values of raw and cooked abalone.

	Springiness		Cohesiveness		Resilience	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Control	0.929 ± 0.137 aB	1.042 ± 0.080 aA	0.592 ± 0.125 aB	0.762 ± 0.044 aA	0.275 ± 0.027 abB	0.408 ± 0.037 aA
5-100	0.843 ± 0.115 aB	1.041 ± 0.123 aA	0.599 ± 0.105 aB	0.769 ± 0.074 aA	0.260 ± 0.062 bB	0.407 ± 0.029 aA
10-100	0.864 ± 0.147 aB	1.004 ± 0.079 aA	0.592 ± 0.142 aB	0.757 ± 0.060 aA	0.255 ± 0.047 bB	0.410 ± 0.026 aA
5-300	0.805 ± 0.115 aB	1.020 ± 0.077 aA	0.633 ± 0.065 aA	0.736 ± 0.056 aA	0.271 ± 0.038 bB	0.413 ± 0.035 aA
10-300	0.876 ± 0.093 aB	1.220 ± 0.769 aA	0.626 ± 0.087 aB	0.746 ± 0.055 aA	0.343 ± 0.104 aB	0.413 ± 0.031 aA

Each value is the mean ± standard deviation (n=12). Values not sharing a lowercase letter are significantly different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test) except for cooked springiness which was analyzed by Kruskall-Wallis (Mann-Whitneyy post-hoc test). Values not sharing an uppercase letter are significantly different within rows for grouped columns (Springiness, Cohesiveness, Resilience), analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test) except for cohesiveness which was analyzed by ANOVA (Tukey's HSD post-hoc test).

The lack of differences among raw treatments is supported by the results reported in chapter 2 at pressures up to 300 MPa for 5 min, as well as a similar HPP abalone study in which texture was not affected by HPP at 550 MPa for 5 min (Briones-Labarca and others 2012). In chapter 2, the firmness value for the post-rigor foot control was 7.705 N while in the present study the firmness value was 3.914 N. While the method used in chapter 2 was identical to the one in the present study, the sample plug size increased in height from 7 mm to 10 mm which, combined with a different batch of abalones, may have contributed to the difference in recorded force. Resilience of the raw 10-300 samples was significantly higher than the other raw treatments with the exception of the raw control. The lack of significant difference between the raw 10-300 and raw control treatments suggest the difference is not due to treatment effect, supported by the lack of difference among cooked treatments for resilience. TPA may not be the best tool to evaluate abalone texture, even if it emulates chewing. Sensory evaluation using a trained panel would be an excellent way to corroborate texture data, and exploration of different instrumental texture analyses such as shear or breaking force may be a better approach to decrease variability.

Cooked samples were significantly ($p<0.05$) more chewy, more springy, and more resilient than raw samples across all treatments (Tables 3.6 and 3.7). Firmness of cooked samples was not significantly different from raw samples except for the 5-300 treatment which was less firm in raw meat than cooked meat. Cohesiveness values of cooked samples were significantly lower than in raw samples for all treatments except the 5-300 treatment. Firmness values of samples ranged from 3.914 N to 7.852 N (raw) to 6.677 N to 10.337 N (cooked). Chewiness values of samples ranged from 1.593 to 3.697 (raw) to

4.861 to 8.378 (cooked). Springiness values of samples ranged from 0.805 to 0.929 (raw) to 1.004 to 1.220 (cooked). Cohesiveness values of samples ranged from 0.592 to 0.633 (raw) to 0.736 to 0.769 (cooked). Resilience values of samples ranged from 0.255 to 0.343 (raw) to 0.407 to 0.413 (cooked). The slight, but significant, 1% water loss during boiling, despite a lack of difference in water holding capacity, may have contributed to the increase in TPA parameters of cooked meat compared to raw due to dehydration of the proteins as a result of denaturation.

Boiling for 3 h has been reported to tenderize abalone meat due to denaturation of myofibrillar proteins as well as gelatinization and subsequent solubilization of collagen fibers (Hatae and others 1996, Gao and others 2002). Other researchers boiled abalones for 15-360 min and reported increased tenderness after boiling only 15 min, with an internal temperature of the meat reported to be 98°C (Hatae and others 1996). In contrast, only one study comparing raw and cooked abalone meat found toughening after boiling. In that study, abalone foot steaks were heated in a water bath at 75ºC for 50 min and the firmness increased from 26.50 N to 37.67 N due to cooking (Sales and others 1999). The firmness values of the cooked abalones in this study were substantially lower than in that study, likely due to the fact the abalones used were wild caught not farmed, were a different species (*Haliotis midae* compared to *H. rufescens*), and were more than six times larger (492 g compared to 80 g), with a presumed much higher collagen concentration due to animal age.

Collagen has been reported to gelatinize at 60°C in mammalian meat, compared to 27.4°C to 33.6°C in fish (Sikorski and others 1985, Cross 1986). The collagen in abalone has been reported to gelatinize at 28°C, so while the internal temperature of the

abalone meat in this study reached 41°C to 66°C after only 15 s, it is plausible that the process of gelatinization was not instantaneous and temperature alone is not enough to cause a conformational change in the collagen (Kimura and Kobota 1968). While the cooked meat had higher TPA parameter values than raw meat, there were no differences within treatments suggesting that HPP did not affect the texture of subsequently cooked abalone meat, which was the primary question in this study.

3.3.5. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to visualize any changes within raw and cooked treatments as well as across treatments. As expected, the fibers in the cooked treatments appeared to be denatured compared to those in the raw images. The denaturation is especially easy to see in the control (Figure 3.1) and 5-100 (Figure 3.2) images, which show distinct fibers in raw muscle and clumped fibers once cooked. In contrast, the 10-100 (Figure 3.3), 5-300 (Figure 3.4), and 10-300 (Figure 3.5) treatment images show less distinction between raw and cooked treatments. The increase in processing time for the 10-100 treatment and the increase in pressure of the 5-300 and 10-300 treatments likely caused denaturation of the proteins before cooking, making visible changes in muscle ultrastructure less apparent.

Figure 3.1. Scanning electron micrographs of the control treatment. Control raw (a) and cooked (b) abalone foot meat taken at 1000x. Scale bars represent 20 µm.

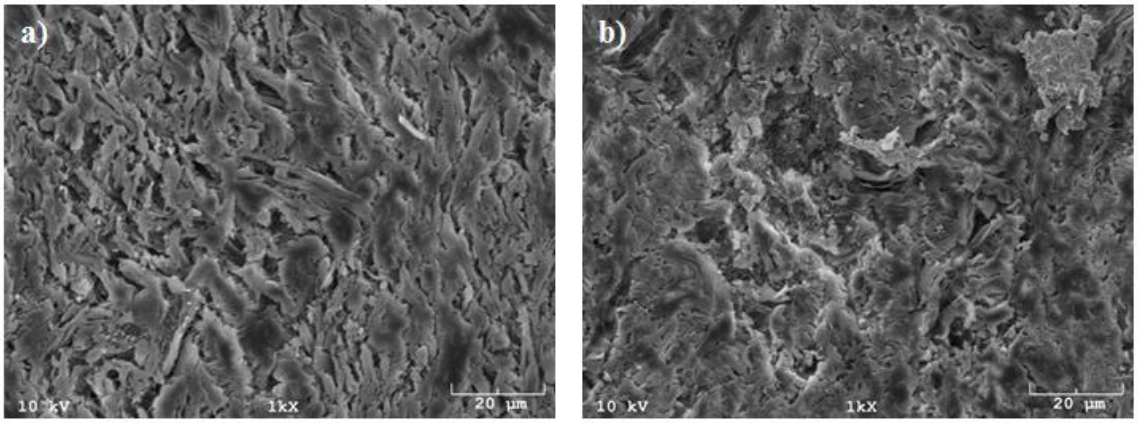


Figure 3.2. Scanning electron micrographs of the 5-100 treatment. Raw (a) and cooked (b) abalone foot meat taken at 1000x. Scale bars represent 20 µm.

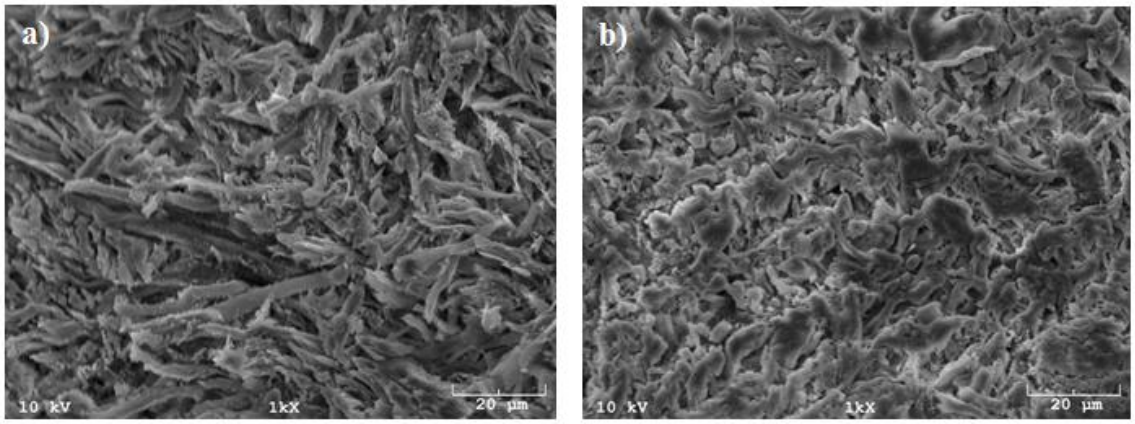


Figure 3.3. Scanning electron micrographs of the 10-100 treatment. Raw (a) and cooked (b) abalone foot meat taken at 1000x. Scale bars represent 20 µm.

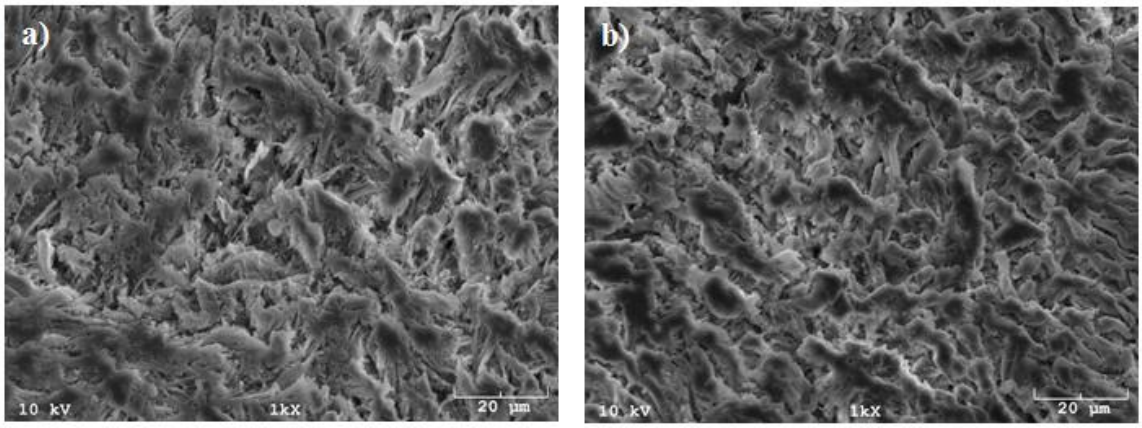


Figure 3.4. Scanning electron micrographs of the 5-300 treatment. Raw (a) and cooked (b) abalone foot meat taken at 1000x. Scale bars represent 20 µm.

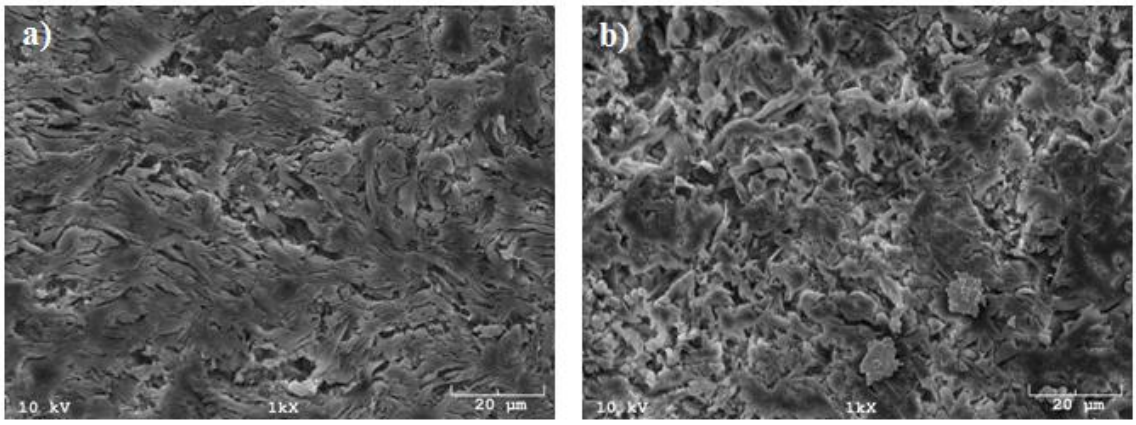
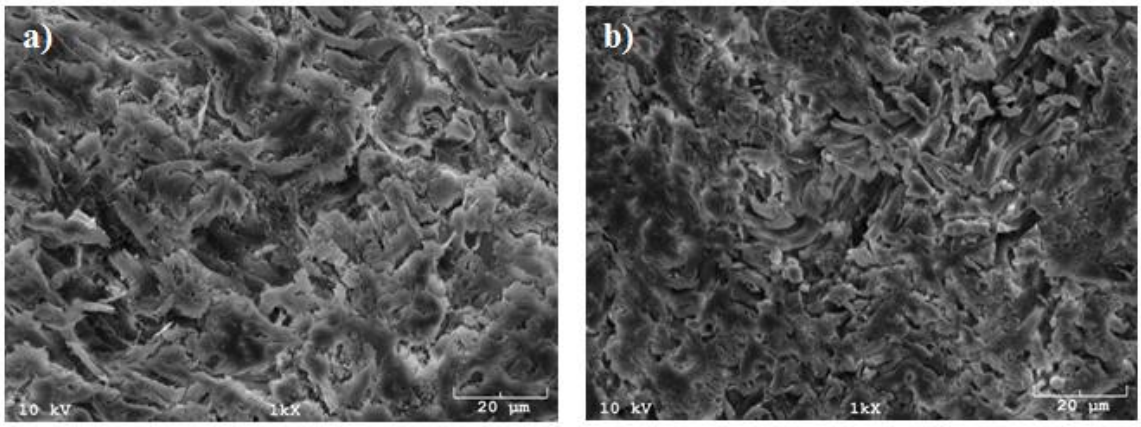


Figure 3.5. Scanning electron micrographs of the 10-300 treatment. Raw (a) and cooked (b) abalone foot meat taken at 1000x. Scale bars represent 20 µm.



3.3.6. Shucking Evaluation

The ease of shucking appeared to improved as HPP time and pressure increased. The control required the greatest amount of force to remove the abalone from the shell, as expected (Table 3.8). Additionally, a metal spoon was needed for the control compared to the processed abalones which were removed by hand. For both the 100 and 300 MPa treatments, shucking ease increased by one point from 5 min to 10 min (Table 3.8). There was no detectable difference between the 10-100 and 5-300 treatments, however,

the 10-300 treatment was two points lower than the 5-100 treatment and three points lower than the control. HPP at all parameters allowed the meats to be shucked by hand without the aid of a metal spoon, and may be able to reduce the time spent manually shucking while also reducing potential damage to the adductor muscle and viscera.

Table 3.8. Shucking evaluation scores.

Treatment	Score
Control	5
5-100	4
10-100	3
5-300	3
10-300	2

3.4. Conclusions

The results of this study demonstrated that subsequently cooked HPP abalone meat had increased whiteness as processing pressure increased, but HPP did not affect the texture of the meat compared to cooked non-HPP abalone. Boiling abalone meat for 15 s allowed internal temperatures to exceed those reported for abalone collagen gelatinization, however boiled meat was significantly tougher compared to raw meat. Scanning electron microscopy images revealed obvious differences in ultrastructure in the raw control and raw minimally processed treatments compared to cooked counterparts, and those differences decreased as processing intensity increased. Shucking of abalones may be improved by HPP, with an inversely proportional relationship observed between increased processing parameters (pressure and time) and force required to remove the abalone meat from its shell. Further investigation into the role of collagen and collagen solubility on abalone texture is warranted, as well as a more robust

evaluation of shucking force and time reduction through the use of HPP. In summary, HPP is a promising process for the abalone industry since it did not negatively affect the texture of abalone even after cooking, and improved the whiteness of subsequently cooked abalone meat.

CHAPTER 4

SHELF-LIFE EVALUATION OF POST-RIGOR HIGH PRESSURE PROCESSED FARM-RAISED ABALONE (*Haliotis rufescens*)

4.1. Objectives

Abalones, like many shellfish, are commonly sold live or frozen due to the very short shelf-life of the shucked meats. Freezing can extend the shelf-life of abalone meat, but may negatively affect texture due to effects of ice crystal formation, such as protein denaturation and dehydration (Strasburg and others 2008). High pressure processing (HPP) has been shown to extend the shelf-life of beef, fish such as red mullet, and shellfish such as shrimp, clams, squid, and oyster (He and others 2002, Fernández and others 2007, Büyükcan and others 2009, Briones and others 2010, Erkan and others 2010, Gou and others 2010, Sánchez-Basurto and others 2011).

HPP has only recently been explored for shelf-life extension of abalone meat, and pressures of 500-550 MPa were found to be capable of extending shelf-life of abalone to 60-65 days (Briones and others 2010, Briones-Labarca and others 2012). HPP abalone texture declined significantly during storage due to proteolytic enzyme activity, however, the color of HPP abalone during storage was found to be stable for 45 days, but values decreased after that (Briones-Labarca and others 2012). While the microbial and biochemical changes in the HPP abalone meat suggested a substantial shelf-life of over 60 days, the decline in the physical quality of the abalone meat cannot be ignored and may in fact be a primary limiting factor from a consumer stand-point, even before freshness indices are exceeded.

The effect of pressures below 500 MPa on abalone shelf-life has not been evaluated, but the observational study conducted in chapter 2 suggests that a 1.5 log reduction in bacterial counts may be achieved at as low as 100 MPa for 1 min. Additionally, the effect of lower pressures on texture and color over time have not been evaluated. The objectives of this study were to assess the effects of HPP pressures less than 500 MPa at different times on the quality of raw refrigerated abalone meat over 35 days at 2ºC by 1) biochemical assays, 2) microbiological assays, and 3) physical quality (texture, color, and microstructure) analyses.

4.2. Materials and Methods

The study had a 2x2 factorial design with two processing pressures (100 and 300 MPa), two processing times (5 and 10 min), and an unprocessed control for a total of five treatments. For each treatment, abalones were divided categorically into microbiological or biochemical analyses. The shelf-life evaluation was conducted over 35 days at 2ºC. Microbiological analyses were conducted initially and every seven days (six total analyses), while biochemical analyses were conducted initially and every five days (eight total analyses). Whole, shucked abalones (n=3) were used for each time point for each treatment, totaling 90 for the microbiological analyses (3x6x5) and 120 for the biochemical analyses (3x8x5). Abalone foot (n=12) was used for texture and color analyses. Reagents were analytical grade and were purchased from Fisher Scientific (Waltham, MA) unless otherwise noted. Microbiological analyses began one day after processing. Biochemical analyses began two days after processing. Initial and final texture and color analyses were conducted four and 35 days, respectively, after processing.

4.2.1. Processing

Live, July-harvested, farm-raised abalones (*Haliotis rufescens*) (n=233) (The Abalone Farm, Cayucas, CA) were divided according to the experimental design. The in-shell abalone (n=117) mean weight was 80.2 g ± 10.2 and the mean shell length was 82.7 mm ± 6.7. Abalones had not been fed for a minimum of 5 days prior to shucking. Abalones were shucked, eviscerated, and scrubbed to remove the epipodium pigment. Shucked meats were loosely packed 20 per bag (Winpak, Films Inc., Senoia, GA) and stored at 1°C 16-21 h to allow mucus production to cease. Following that time period, the abalones were rinsed and packed 6 per bag at 99% vacuum (Model UV550, Koch Industries, Wichita, KS). Bags were kept on ice during transport to the Natick Soldier Research, Development, and Engineering Center (Natick, MA). Controls were kept in ice in coolers in the Matthew Highlands Pilot Plant (Orono, ME).

A 1 L HPP unit (Engineering Pressure Systems Inc., Haverhill, MA) was used and processing was randomized among treatments. The temperature of the vessel during pressurization ranged from 20°C-25°C. Hydraulic fluid (20:1 water:Hydrolubric 120-B (Houghton International Inc., Norristown, PA)) was used to achieve hydrostatic pressure. The come-up time ranged from 3 min to 4.5 min and depressurization was immediate. Following processing, all treatments, including the controls, were aseptically re-vacuum packed, three abalones per bag, at 99% in 10,000 oxygen transmission rate (OTR) cornstarch dusted bags (Cryovac, Bethlehem, PA) and stored at 1-2°C for the duration of the study.

4.2.2. Microbiological Analyses

Microbiological analyses were conducted every 7 days to quantify mesophilic and psychrophilic bacteria. There were three abalones per treatment per time period, and each abalone was evaluated separately. Individual whole shucked abalones were aseptically placed in individual filtered stomacher bags with sterile 0.1% bactopeptone (BD Diagnostics, Sparks, MD) (1:10 w/w ratio). The bags were mixed for 2 min using a BagMixer 400 (Model P, SpiralBiotech, Advanced Instruments, Norwood, MA). Serial dilutions of 1:10, 1:100, and 1:1000 were used for both aerobic plate counts (APC) and psychrophilic plate counts (PPC), increasing the dilution weekly as needed. Aliquots of 1 mL of each dilution were dispensed on Aerobic Plate Count PetriFilm (3M, St. Paul, MN) in duplicate, and the films were incubated (model 150, Labline Instruments, Melrose Park, IL) for 2 days at 35°C for APC and 4°C for 5 days for PPC. Averages from the duplicate films were used to compare results. PPC analysis was terminated after 12 days after no growth was observed. APC results were transformed logarithmically for all data analyses.

4.2.3. Biochemical Analyses

There were three abalones per treatment per time period, and each abalone was assessed individually. Abalones were assessed every 5 days for biochemical shelf-life indicators. The individual whole abalones were chopped and homogenized, and subsamples were taken for each of the three biochemical analyses (total volatile base nitrogen, K-value, and biogenic amines). Duplicate analyses from the individual abalones were not possible due to the small size of the abalones.

4.2.3.1. Total Volatile Base Nitrogen

A microblender (Waring, Stamford, CT) was used to homogenize 15 g chopped sample in 30 mL 7.5% trichloroacetic acid (TCA) for 30 s. The homogenate was centrifuged (model 5430, Eppendorf, Hamburg, Germany) for 20 min at 1,878 *g*. The filtrate was stored at 4°C until distillation at which point 15 mL were added to the micro-Kjeldahl distillation apparatus with 4 mL 10% NaOH. The blank was made with 20 mL TCA and 6 mL DI water. Indicator was made with 2:1 0.2% methyl red:0.2% methylene blue, both in ethanol. Samples were distilled into 15 mL 4% boric acid with 8 drops of indicator to a final volume of 40 mL. Distillate was titrated using 0.1 N HCl to a constant purple color. Volume (mL) of HCl required was related to quantity of TVBN in the sample, and a standard made with ammonium sulfate and trimethylamine-HCl was used at a concentration of 4.26% nitrogen/mL to check accuracy of the method. The amount of TVBN (mg/100 g) in each sample was calculated by multiplying (volume (mL) of HCl x normality of HCl x molecular weight of nitrogen) x (volume (mL) of extraction fluid/volume (mL) of fluid added to distillation apparatus) x (100/original weight (g) of sample).

4.2.3.2. K-Value

K-value analysis was conducted following the methods of Ryder (1985) and Özogul and others (2000). A microblender (Waring, Stamford, CT) was used to homogenize 5 g chopped sample in 25 mL 6% perchloric acid (Sigma-Aldrich, St. Louis, MO) for 30 s. The homogenate was centrifuged for 10 min at 1,057 *g* (model 5430, Eppendorf, Hamburg, Germany). A 10 mL aliquot of the supernatant was immediately

neutralized to pH 6.5-6.8 with 1 M KOH. The neutralized supernatant was allowed to stand 30 min at 0°C to precipitate potassium perchlorate crystals prior to filtration through Whatman #1 filter paper. Filtrate was brought to 20 mL with DI water and stored in a plastic vial at -80°C for 4 months until HPLC analysis.

The standards adenosine triphosphate (ATP) (Alfa Aesar, Ward Hill, MA), adenosine diphosphate (ADP) (Sigma-Aldrich, St.Louis, MO), adenosine monophosphate (AMP) (Sigma-Aldrich, St.Louis, MO), inosine monophosphate (IMP) (Sigma-Aldrich, St. Louis, MO), inosine (INO) (Alfa Aesar, Ward Hill, MA), and hypoxanthine (HX) (Alfa Aesar, Ward Hill, MA) were mixed into standard cocktails (0.004-0.250 mM) to quantify nucleotides in the samples. Samples and standards were randomly injected on the HPLC (1100/1200, Agilent Technologies, Santa Clara, CA) at a volume of 0.5 uL per injection. The mobile phase was 0.06 M K_2HPO_4 and 0.04 M KH_2PO_4. A Gemini C6 column (5µm x 4.6 mm x 250 m) (Phenomenex, Torrance, CA) was used to elute nucleotides at a flow rate of 2 mL/min at ambient temperature. The UV detector was set at 254 nm and ChemStation software (Agilent Technologies, Santa Clara, CA) was used to calculate peak areas. Nucleotides eluted in decreasing order of polarity: IMP, ATP, ADP, AMP, HX, INO. Concentrations (mM) were determined by using the equation of the line of the standard curves. K-value was calculated by [(INO+HX)/(ATP+ADP+AMP+IMP+INO+HX)]*100.

4.2.3.3. Biogenic Amines

A microblender (Waring, Stamford, CT) was used to homogenize 5 g chopped abalone with 20 mL 6% trichloroacetic acid. The homogenate was centrifuged at 11,180

g (model J2-21, Beckman Coulter, Brea, CA) for 10 min at 4°C then filtered through Whatman #1 filter paper and brought to 50 mL with DI water (Özogul and others 2006). A 20 mL aliquot of each sample was stored in plastic vials for 7-8 months at -80°C. Samples were thawed at 4°C prior to preparation for HPLC by benzoylation, the purpose of which is to attach a benzoyl ring to the amines to make them detectable by HPLC. The standards selected were agmatine (Alfa Aesar, Ward Hill, MA), cadaverine (Sigma-Aldrich, St. Louis, MO), histidine (ICN Biomedicals Inc., Aurora, OH), putrescine (Acros Organics, Fairlawn, NJ), and tyramine (Alfa Aesar, Ward Hill, MA) which have been previously identified in snail meat (Chotimarkorn and others 2010). The standards were combined into a standard cocktail (0.0005-0.5 mg/mL) to quantify selected biogenic amines in the samples, reported as mg/100 g meat.

A 2 mL aliquot from each standard concentration and sample was placed in individual test tubes with 1 mL 2 M NaOH and 1 mL 2% benzoyl chloride (Acros Organics, Fairlawn, NJ). The solution was left at ambient temperature for 30 min, then the benzoylation was stopped with 2 mL saturated NaCl solution (Chotimarkorn and others 2010). Extraction of the biogenic amines was accomplished by two diethyl ether washes. The ether was evaporated under a constant stream of nitrogen and the residue was brought to 1 mL with acetonitrile, flushed with nitrogen, and stored at -15°C until HPLC analysis.

Samples and standards were randomly injected on the HPLC (1100/1200, Agilent Technologies, Santa Clara, CA) at a volume of 5 uL per injection. The mobile phase was 75% 0.1% trifluoroacetic acid (Acros Organics, Fairlawn, NJ) and 25% acetonitrile. A Nucleosil C18 column (5µm x 4.6 mm x 150 m) (Phenomenex, Torrance, CA) was used

to elute amines at a flow rate of 1 mL/min at ambient temperature. The UV detector was set at 210 nm and ChemStation software (Agilent Technologies, Santa Clara, CA) was used to calculate peak areas. Standards eluted in the order of tyramine, agmatine, putrescine, and cadaverine. Histidine did not elute after 1 hr, possibly due to age of standard.

4.2.4. Color and Texture Analyses

Color and texture analyses were conducted initially and at the conclusion of the shelf-life study (day 35). For initial analyses, 12 abalones were analyzed per treatment. For the Day 35 assessments, three abalones were analyzed per treatment. At both time points, whole abalones were sliced horizontally below the base of the adductor muscle to separate it from the foot muscle. A digital caliper was used to measure 20x20x10 mm square plugs which were cut from the center of each foot muscle on the ventral side. Plugs were stored in 7.5 x 12.5 cm WhirlPak (Nasco, Fort Atkinson, WI) bags at 4°C until same-day color and texture analyses were conducted.

4.2.4.1. Color

Colorimetric analyses were performed on the abalone muscle plugs (n=12) using a colorimeter (LabScan XE, Hunter Labs, Reston, VA). The Hunter L, a, b values were recorded by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs, Reston, VA). Whiteness was calculated by the equation $W=100-((100-L)^2+a^2+b^2)^{0.5}$. The colorimeter was standardized using white and black tiles for a port size of 25.4 mm

and an area view of 30.5 mm. After the initial color reading, each plug was rotated 120º. The rotation was repeated a final time to achieve three readings, which were then averaged to one value per plug.

4.2.4.2. Texture

Following color analysis, foot plugs were placed on the calibrated texture analyzer platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) ventral side up. The texture analyzer was configured for Texture Profile Analysis (TPA) with a 10 mm cylindrical probe, 5 mm/s test speed, two 30% (3 mm) compressions, and a 5 s gap between compressions. TPA emulates chewing, and can be useful for comparison to the consumer experience (Bourne 2002). Force (N), area (N*s), distance (mm) between peak heights, and time (s) were recorded by the texture analysis software (Exponent 32, version 5,0,6,0 2010, Texture Technologies Inc., Scarsdale, NY) to calculate TPA parameters.

The five parameters evaluated were firmness, springiness, cohesiveness, chewiness, and resilience. Firmness is resistance to compression, determined by the peak force (N) of the first compression (Bourne 2002). Springiness represents how well a product springs back after compression, calculated by dividing the distance to peak force of the second compression by the distance to the peak force of the first compression (Bourne 2002). Cohesiveness corresponds to how a product resists second compression compared to the first, a unitless number calculated by dividing the work area of the first compression by the work area of the second compression (Bourne 2002). Chewiness characterizes elastic resistance of a solid food, a unitless number calculated by

multiplying hardness, cohesiveness, and springiness (Bourne 2002). Resilience describes the immediate springiness as the probe is withdrawn between "bites", a unitless number calculated by dividing the area of the withdrawal of the first compression by the area of the first compression (Bourne 2002).

4.2.5. Statistical Analyses

Data were analyzed using SYSTAT 12 (Systat Software, Chicago, IL) for one-way analysis of variance (ANOVA) for all one-level (treatment) analyses, and multi-way ANOVA to assess multiple independent variables. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses. The Shapiro-Wilk normality test and Levene equality of variances test were used to assess data prior to analyses. In cases where data did not satisfy either normality or homogeneity, they were evaluated non-parametrically using Kruskal-Wallis. Mann-Whitney was selected for non-parametric post-hoc analyses. Pearson's Correlation with a Bonferroni Correction was used to evaluate correlations among shelf-life results, and between pressure and shelf-life results. For all statistics, a significance level of $p<0.05$ was selected.

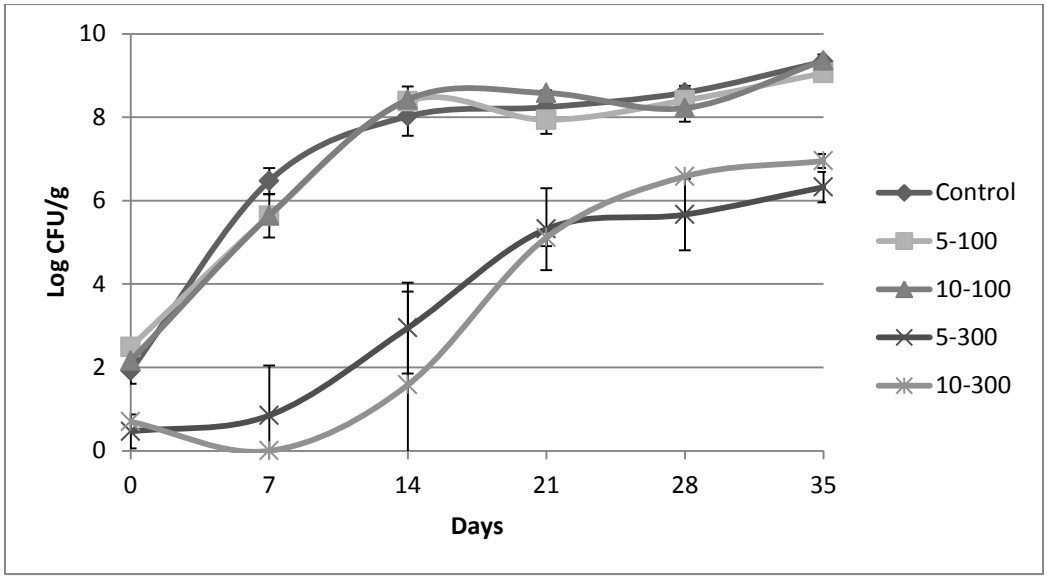
4.3. Results and Discussion

4.3.1. Microbiological

Aerobic plate counts (APC) increased significantly ($p<0.05$) over time for all treatments, but were significantly higher for the control and 100 MPa treatments compared to the 300 MPa treatments (Figure 4.1). Colony forming units (CFU) were not detected on psychrophilic plates in any treatment. Freshness of seafood is generally limited to 10^6 colony forming units (CFU)/g, with sensory rejection occurring at 10^8

CFU/g, or before depending on the food product, due to the organoleptic changes caused by the specific spoilage organism (Gram and Huss 1996). The most common microorganisms found on seafood are *Shewanella* spp. and *Pseudomonas* spp. which are described as psychrotolerant, or capable of growing at refrigerated temperatures but with optimum growth temperatures nearer mesophilic ranges (Gram and Dalgaard 2002). The presence of psychrotolerant bacteria on abalone raised in California is expected due to its native water temperature ranging from 13-17ºC (NODC 2013).

Figure 4.1. Aerobic plate counts of abalone during refrigerated storage.



There were no significant differences among treatments at Day 0, with counts ranging from 0.47 log CFU/g (5-300) to 2.49 log CFU/g (5-100) (Table 4.1). Unprocessed abalone meat has been reported to have initial counts of 1.3 log CFU/g, with a count reduction of nearly 1 log in abalone processed at 500 or 550 MPa for 3, 5, or 8 min (Briones and others 2010). The lower pressures used in the present study were not enough to inactivate spoilage organisms entirely, as evidenced by the detected microbial

counts in the processed abalone treatments on Day 0, however, similar to the findings of Briones and others (2010) there was a microbial reduction of over 1 log from the control (1.92 log CFU/g) compared to the 5-300 and 10-300 treatments (0.47 and 0.70 log CFU/g) even though it was not statistically significant. The 1 log reduction was anticipated based on the observational microbiology evaluation conducted in chapter 2, in which a 1.5 log reduction was observed after processing abalone for 1 min at 100 MPa.

In contrast, by Day 7 the 300 MPa treatments had significantly lower APC (<1 log CFU/g) than the 100 MPa treatments (>5 log CFU/g) and the control (>6 log CFU/g), suggesting an impairment of growth and a slowing of the exponential phase due to the higher pressure. By Day 14, the control and 100 MPa treatments exceeded 8 log CFU/g, while the 300 MPa treatments remained below 7 log CFU/g for the duration of the 35 day refrigerated shelf-life evaluation. These results suggest that 300 MPa for 5 or 10 min is sufficient to extend the shelf-life of abalone 5 times and 4 times, respectively, that of the unprocessed control. A refrigerated (4ºC) shelf-life of 65 days was achieved with pressures of 500 and 550 MPa for 3, 5, and 8 min, however, the control also remained below 106 CFU/g for the 65 days and counts of processed abalones did not increase after day 25 which suggests an extended stationary phase as opposed to immediate destruction of microbes due to pressure (Briones and others 2010). In contrast, in the current study, APC in all five treatments continued to increase with time suggesting the stationary phase had not been reached. Longer shelf-life evaluations at the 300 MPa level would inform the final microbial shelf-life of HPP abalone. Sensory evaluation with a trained panel would also need to be conducted to verify acceptance at each time point since microbial counts alone cannot determine freshness or acceptability.

Table 4.1. Aerobic plate counts (log CFU/g) of abalone during refrigerated storage.

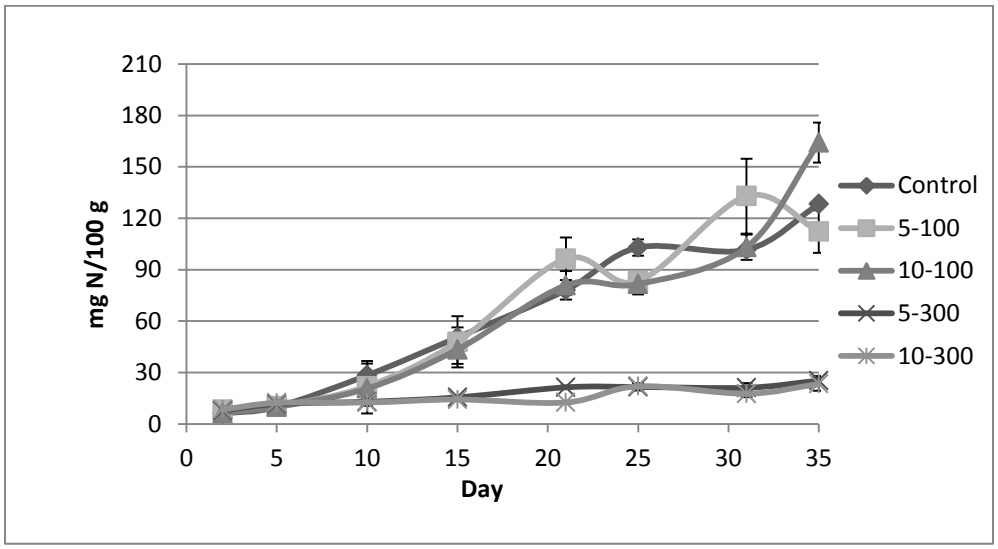
Day	Control	5-100	10-100	5-300	10-300
0	1.92 ± 0.32 abC	2.49 ± 0.03 aB	2.16 ± 0.10 abD	0.47 ± 0.40 bC	0.70 ± 0.00 bB
7	6.47 ± 0.31 aBC	5.63 ± 0.52 aB	5.64 ± 0.18 aC	0.85 ± 1.20 bC	0.00 ± 0.00 bB
14	8.02 ± 0.46 aABC	8.38 ± 0.17 aAB	8.42 ± 0.32 aB	2.95 ± 1.09 bBC	1.58 ± 2.24 bB
21	8.24 ± 0.28 abABC	7.94 ± 0.34 abAB	8.58 ± 0.06 aB	5.32 ± 0.98 bAB	5.11 ± 0.20 bAB
28	8.59 ± 0.16 aAB	8.40 ± 0.18 aAB	8.22 ± 0.32 aB	5.67 ± 0.86 bAB	6.58 ± 0.00 bAB
35	9.33 ± 0.02 aA	9.06 ± 0.06 aA	9.36 ± 0.15 aA	6.32 ± 0.36 cA	6.95 ± 0.17 bA

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly ($p<0.05$) different within rows, analyzed by ANOVA (Tukey's HSD post-hoc test) except for days 0 and 21 for which Kruskall-Wallis (Mann-Whitney post-hoc test) was used. Values not sharing an uppercase letter are significantly ($p<0.05$) different within columns, analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test) for all treatments except 10-100 and 5-300 for which ANOVA (Tukey's HSD post-hoc test) was used.

4.3.2. Total Volatile Base Nitrogen

Total volatile base nitrogen (TVBN) values increased significantly ($p<0.05$) with time for all treatments, but were significantly higher for the control and 100 MPa treatments than for the 300 MPa treatments (Figure 4.2). TVBN represents the quantity of non-protein nitrogen, such as nucleotides, sulfur containing amino acids, and trimethylamine oxide, available for bacteria to convert to volatiles such as trimethylamine, methylmercaptan, and ammonia among others (Gram and Huss 1996).

Figure 4.2. Total volatile base nitrogen concentrations of abalone during refrigerated storage.



TVBN concentrations of 25-35 mg nitrogen (N)/100 g are considered the upper limits of freshness for seafood (Pyrgotou and others 2010, Orban and others 2011, Feng and others 2012). Initial TVBN values for this study ranged from 6.03 mg N/100 g (control) to 8.38 mg N/100 g (10-300), but were not significantly different among treatments (Table 4.2). In shelf-life studies of unprocessed abalone, TVBN concentrations increased to maximum values of ~7.5 mg N/100 g over 3.5 days in one

study, and 7.34 mg N/100 g to 20 mg N/100 g over 15 days for two other studies (Watanabe and others 1992, Chiou and others 2002, Siripatrawan and others 2009). Comparatively, at Day 15 in the current study, the control TVBN concentration was 50.51 mg N/100 mg while the 300 MPa treatments were significantly lower, with average concentrations of 15.51 mg N/100 g (5 min) and 14.24 mg N/100 g (10 min).

At the end of the 35 day shelf-life evaluation, the 300 MPa treatments were still below the recommended threshold at 25.27 mg N/100 g (5 min) and 23.77 mg N/100 g (10 min). Abalones processed at 500 MPa for 8 min had TVBN values of only 22.37 mg N/100 g after 60 days at 4ºC, but that value was not different from the TVBN value reported for day 21 of 22.97 mg N/100 g (Briones-Labarca and others 2012). The lack of increase in TVBN values over time suggests a stagnation of bacterial activity, as opposed to a direct effect of processing at 500 MPa, and it is hypothesized that similar values would have been obtained at day 60 in abalones processed at 300 MPa since the values in the present study correspond so closely with those of the abalones processed at 500 MPa.

The control and 100 MPa treatments, however, were 4-6 times higher than the 300 MPa treatments, with TVBN concentrations ranging from 112.29 mg N/100 g (5-100) to 164.12 mg N/100 g (10-100). Unprocessed abalone in a similar study were reported to have TVBN values of 189 mg/100 g after 60 days, again suggesting a stagnation of bacterial activity (Briones-Labarca and others 2012). These higher values for the control and 100 MPa treatments correspond to the significantly higher microbial loads on those treatments, and indicate increased bacterial enzymatic activity as protein and non-protein nitrogen components of the abalone were degraded (Gram and Huss 1996). The 300 MPa treatments were below the freshness threshold for the duration of

the shelf-life study, suggesting that a processing pressure of 300 MPa for 5 or 10 min is sufficient to significantly extend shelf-life of abalone at 2ºC.

Table 4.2. Total volatile base nitrogen concentrations (mg N/100 g) of abalone during refrigerated storage.

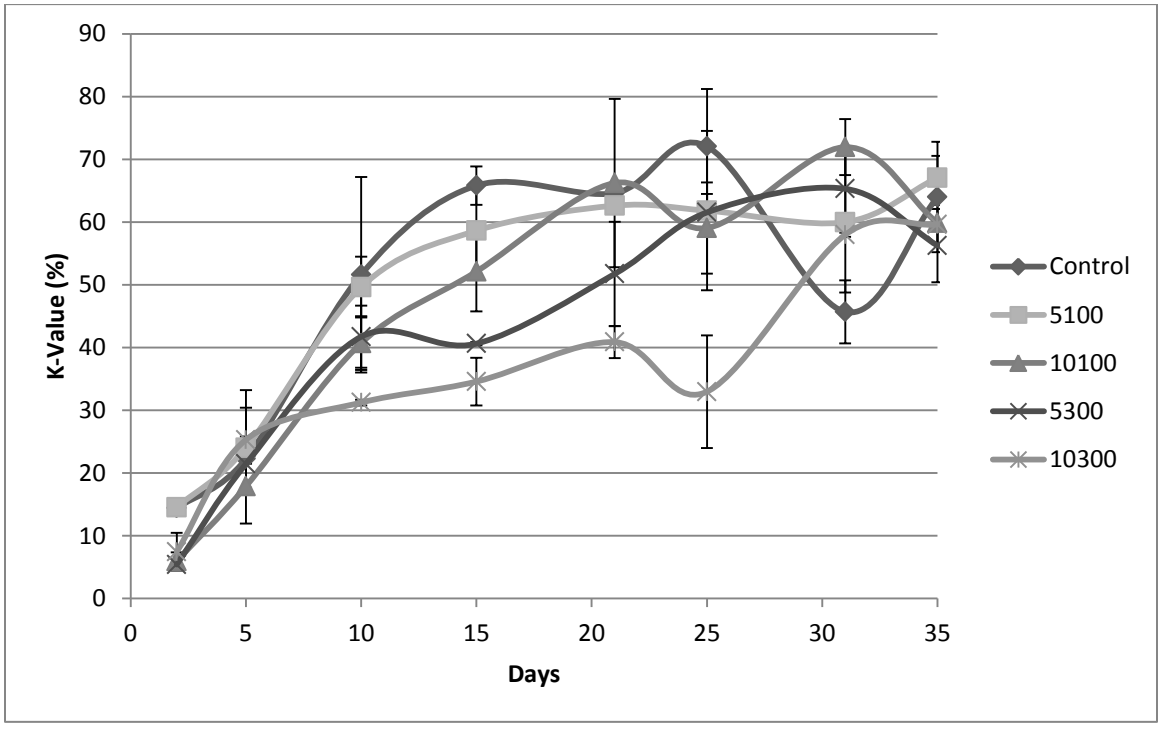
Day	Control	5-100	10-100	5-300	10-300
2	6.03 ± 2.68 aF	8.16 ± 1.49 aD	6.44 ± 1.26 aD	7.75 ± 1.30 aC	8.38 ± 0.85 aC
5	9.94 ± 1.50 aF	10.33 ± 0.75 aD	9.91 ± 0.75 aCD	11.63 ± 1.87 aBC	12.28 ± 2.76 aBC
10	28.35 ± 8.40 aE	21.94 ± 3.94 aCD	20.67 ± 14.56 aCD	12.92 ± 1.31 aBC	12.49 ± 2.00 aBC
15	50.51 ± 5.76 aD	47.90 ± 14.91 aC	43.44 ± 8.39 aC	15.51 ± 1.32 bB	14.24 ± 0.01 bBC
21	78.08 ± 2.68 aC	96.40 ± 12.40 aB	80.87 ± 8.29 aB	21.34 ± 0.93 bAB	12.51 ± 0.74 bBC
25	102.92 ± 4.72 aB	83.21 ± 1.77 bB	81.58 ± 5.98 bB	21.55 ± 2.02 cAB	21.97 ± 1.74 cA
31	101.62 ± 0.68 aB	132.90 ± 21.89 aA	103.10 ± 7.31 aB	21.11 ± 2.70 bAB	17.66 ± 1.96 bAB
35	128.26 ± 2.15 abA	112.29 ± 12.43 bAB	164.12 ± 11.66 aA	25.27 ± 2.75 cA	23.33 ± 3.89 cA

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly ($p<0.05$) different within rows, analyzed by ANOVA (Tukey's HSD post-hoc test). Values not sharing an uppercase letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test).

4.3.3. K-Value

K-values (%) increased significantly ($p<0.05$) over time for all treatments, but were not significantly different among treatments at most evaluation times (Figure 4.3). K-value is a ratio of nucleotide break-down products by enzymatic action, whether autolytic or bacterial (Chang and others 1998). While characteristic K-values are highly species specific as well as dependent on initial quantities of ATP, early work developing K-value as a freshness indicator for fish found that K-values of less than 20% can be considered very fresh, 50% can be considered moderately fresh, and values higher than 70% are not fresh (Saito and others 1959). An additional consideration when quantifying K-value is that it increases faster with bacterial action as opposed to just by autolytic degradation (Gram and Huss 1996).

Figure 4.3. Average K-values of abalone during refrigerated storage.



The initial K-values for the control and the 5-100 treatment were 14.4% and 14.5%, respectively, which was more than twice the level of the 10-100, 5-300, and 10-300 treatments (Table 4.3). By comparison, in a 3.5 day evaluation of abalone held at 5ºC, the time zero K-value was only 2.4% and rose to a maximum of ~13% (Chiou and others 2002). The sharp difference from time zero to only 3.5 days is reflective of how quickly K-value changes over time due to autolytic activity, and in the two days between processing and biochemical evaluation it is likely that values rose in a similar manner.

Comparable to the values reported in this study for Days 10-15, a longer shelf-life evaluation of abalone reported K-values at 5ºC to be ~40% after 15 days (Watanabe and others 1992). By Day 35, K-values ranged from 56.3% (5-300) to 67.1% (5-100), above the 50% moderately fresh indicator. The control and 100 MPa treatments could be considered moderately fresh through Day 10, while the 300 MPa treatments could be considered moderately fresh through Day 21 (5-300) and Day 25 (10-300). The values for the 10-100 and both 300 MPa treatments were significantly lower than the control and 5-100 treatments only for Day 2 and Day 15, suggesting that high pressure may have delayed nucleotide degradation initially, but it did not have a significant effect on K-value over 35 days. These results suggest that though both microbial loads and TVBN had significant differences due to HPP, that nucleotide degradation of abalone is independent of HPP at the pressures used in this study, which may have been too low to deactivate autolytic enzymes such as ATPase.

Table 4.3. Average K-values (%) of abalone during refrigerated storage.

Day	Control	5-100	10-100	5-300	10-300
2	14.4 ± 1.1 aC	14.5 ± 1.1 aB	6.0 ± 1.4 bD	5.4 ± 0.0 bC	7.4 ± 3.0 bC
5	22.3 ± 0.8 aBC	24.0 ± 6.4 aB	17.9 ± 5.9 aD	21.4 ± 4.4 aC	25.3 ± 7.9 aB
10	51.6 ± 15.6 aA	49.6 ± 4.8 aA	40.7 ± 4.3 aC	41.7 ± 4.9 aB	31.2 ± 0.5 aB
15	65.8 ± 3.1 aA	58.6 ± 7.7 aA	52.1 ± 6.3 abBC	40.6 ± 0.2 bcB	34.6 ± 3.8 cB
21	64.6 ± 0.0 aA	62.6 ± 0.8 aA	66.2 ± 13.4 aAB	51.7 ± 8.3 aAB	40.9 ± 2.6 aB
25	72.0 ± 9.2 aA	61.8 ± 12.7 aA	59.0 ± 7.3 aAC	61.5 ± 2.9 aA	33.0 ± 9.0 aB
31	45.7 ± 5.0 bAB	60.0 ± 11.2 abA	71.9 ± 4.5 aA	65.3 ± 6.6 aA	58.0 ± 0.3 abA
35	64.0 ± 8.8 aA	67.1 ± 3.5 aA	59.9 ± 0.5 aAC	56.3 ± 5.8 aAB	59.6 ± 3.2 aA

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly ($p<0.05$) different within rows, analyzed by ANOVA (Tukey's HSD post-hoc test). Values not sharing an uppercase letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test).

4.3.4. Biogenic Amines

The biogenic amines cadaverine and putrescine increased significantly ($p<0.05$) with time for the control and 100 MPa treatments, however were undetected for 35 days in the 300 MPa treatments (Figures 4.4 and 4.5). Agmatine, histamine, and tyramine were undetected in all treatments at all time points. Similarly, histamine and tyramine were not detected in disk abalone stored at 5ºC for 15 days (Watanabe and others 1992). Biogenic amines are low molecular weight bases which are measured as indicators of muscle food freshness and safety due to the harmful toxicological effect they may have if levels are too high (ten Brink and others 1990). Biogenic amines are formed from free amino acids that are decarboxylated by bacterial action, and so co-occur with increases in microbial activity (ten Brink and others 1990). The similarity between the APC figure (4.1) and both biogenic amine figures (4.4 and 4.5) underscores this relationship, with the control and 100 MPa treatments having both higher microbial loads and higher biogenic amine levels.

Figure 4.4. Average cadaverine concentrations of abalone during refrigerated storage.

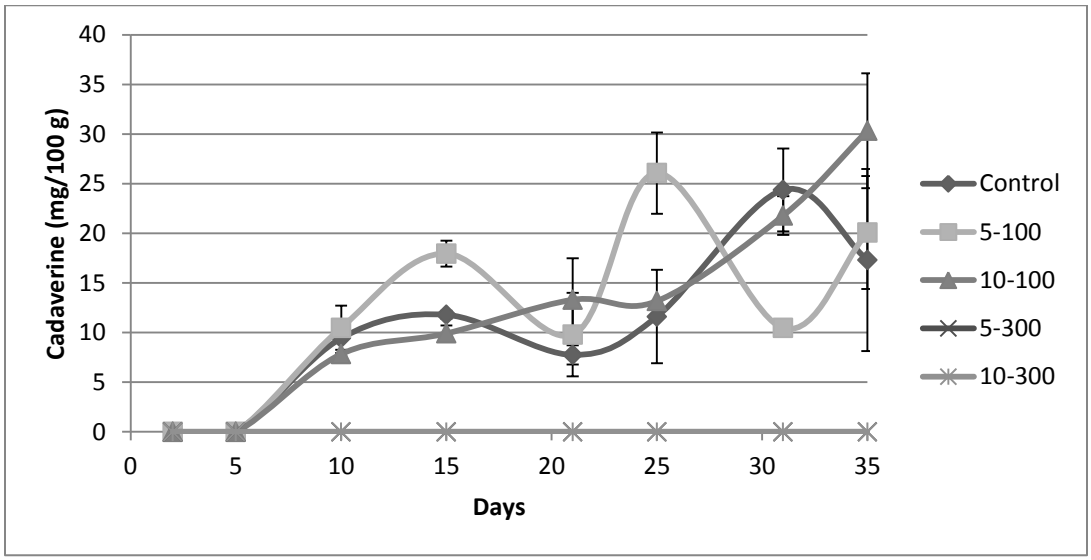
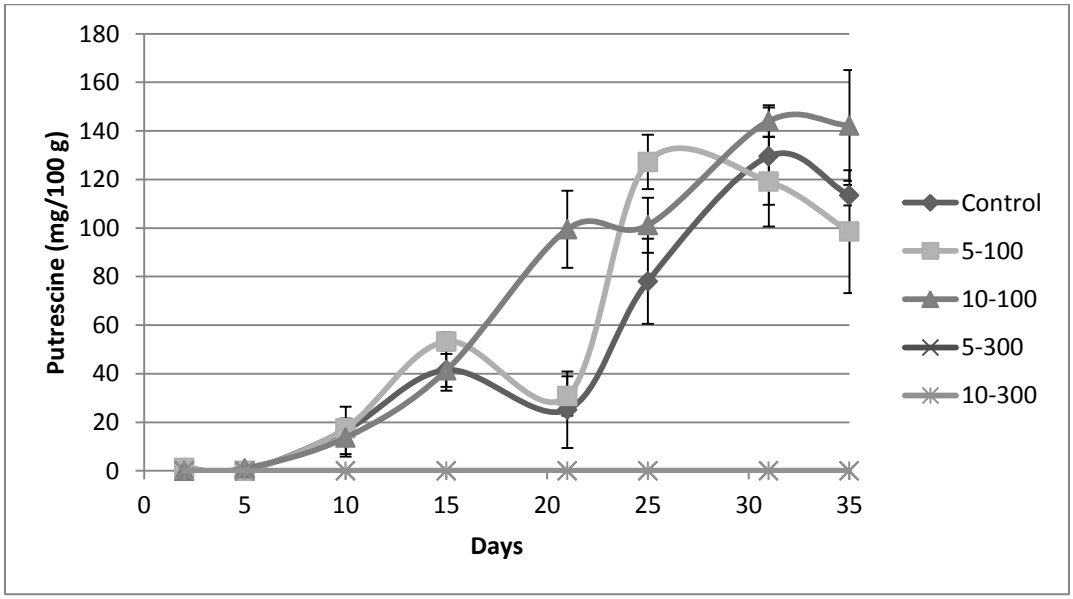


Figure 4.5. Average putrescine concentrations of abalone during refrigerated storage.



The absence of quantified biogenic amines in the 300 MPa treatments suggests that while there was bacterial growth over the 35 days, decarboxylation of free amino acids was not occurring at detectable levels. The absence of biogenic amines in the 300 MPa treatments may also have been due to their significantly lower microbial counts. The control and 100 MPa treatments reached APC of 10^6 by Day 7, which parallels when the biogenic amine production increased from 0.00 mg/100 g to detectable levels of both cadaverine (Table 4.4) and putrescine (Table 4.5). In contrast, microbial counts for the 300 MPa treatments did not increase to the 10^6 level until the end of the shelf-life study, possibly suggesting that microbial activity must hit a critical point before biogenic amine concentrations reach detectable levels. It is also possible that the high pressure used in the 300 MPa treatments damaged the bacteria during processing, slowing growth and decarboxylation, thereby limiting production of biogenic amines even as growth increased with time.

Cadaverine production increased significantly in the control and 100 MPa treatments over time, ranging from 17.31 mg/100 g (control) to 30.33 mg/100 g (10-100) by Day 35, though there were no significant differences among those treatments. Unprocessed abalone was reported to have a cadaverine concentration of 5.7 mg/100 g after 15 days of storage at 5ºC (Watanabe and others 1992). That cadaverine level was lower than the values reported in the current study for Day 15 of 11.78 mg/100 g (control), 17.95 mg/100 g (5-100), and 9.91 mg/100 g (10-100). Other molluscan shelf-life studies have reported cadaverine levels of 6.66 mg/100 g after 7 days for iced snail, ~0.45 mg/100 g after 28 days for partially dried squid, and 11.5 mg/100 g after 10 days for iced scallop (Mackie and others 1997, Chotimarkorn and others 2010, Gou and others

2010). Additionally, the partially dried squid was high pressure processed and the HPP samples had lower cadaverine levels than the control (Gou and others 2010) which was also true in the current study for the 10-100 treatment and both 300 MPa treatments.

Putrescine production was higher than cadaverine for all treatments except the 300 MPa treatments, for which there were no detectable biogenic amines. Putrescine is derived from ornithine (a derivative of arginine), and cadaverine is derived from lysine (ten Brink and others 1990), so it is likely that the abalone meat had higher levels of ornithine than lysine based on the resultant biogenic amine levels. Disk abalone have been reported to contain 36.4 mg lysine/100 g, 8.7 mg ornithine/100 g, and 393.4 mg arginine/100 g (Watanabe and others 1992). The abalones used in the present study may have higher ornithine contents than disk abalone, or it is possible that the large quantities of arginine were converted to ornithine.

In molluscan shelf-life studies, putrescine concentrations were similarly higher than cadaverine concentrations, however, levels were lower than reported in the current study. Putrescine concentrations were 11.4 mg/100 g after 10 days for scallop, 3.4 mg/100 g after 15 days for abalone, and 11.6 mg/100 g after 7 days for snail (Watanabe and others 1992, Mackie and others 1997, Chotimarkorn and others 2010). By comparison, Day 15 putrescine concentrations in the current study were 41.5 mg/100 g (control), 53.3 mg/100 g (5-100), and 41.4 mg/100 g (10-100) suggesting active decarboxylation of ornithine by bacterial populations during storage at 2ºC.

Based on the absence of agmatine, cadaverine, histamine, putrescine, and tyramine in both 300 MPa treatments, it appears that HPP at 300 MPa for 5 or 10 min is

sufficient to prevent biogenic amine production compared to unprocessed abalone and abalone processed at 100 MPa for 5 or 10 min. The maximum total concentration of biogenic amines in a food product is recommended to be 75-90 mg/100 g (Ladero and others 2010), which was reached by Day 21 for the control and 100 MPa treatments, however, the 300 MPa treatments would be considered safe for the full 35 days.

Table 4.4. Average cadaverine concentrations (mg/100 g) of abalone during refrigerated

Day	Control	5-100	10-100	5-300	10-300
2	0.00 ± 0.00 aB	0.00 ± 0.00 aB	0.00 ± 0.00 aC	0.00 ± 0.00 aA	0.00 ± 0.00 aA
5	0.00 ± 0.00 aB	0.00 ± 0.00 aB	0.00 ± 0.00 aC	0.00 ± 0.00 aA	0.00 ± 0.00 aA
10	9.43 ± 1.78 aAB	10.48 ± 2.23 aAB	7.83 ± 0.12 abBC	0.00 ± 0.00 bA	0.00 ± 0.00 bA
15	11.78 ± 0.40 bA	17.95 ± 1.31 aA	9.91 ± 0.81 cABC	0.00 ± 0.00 dA	0.00 ± 0.00 dA
21	7.75 ± 0.96 abAB	9.79 ± 4.21 abAB	13.30 ± 4.17 aABC	0.00 ± 0.00 bA	0.00 ± 0.00 bA
25	11.61 ± 4.70 abAB	26.07 ± 4.09 aA	13.17 ± 0.03 abABC	0.00 ± 0.00 bA	0.00 ± 0.00 bA
31	24.37 ± 4.18 aA	10.46 ± 0.72 abAB	21.78 ± 1.96 aAB	0.00 ± 0.00 bA	0.00 ± 0.00 bA
35	17.31 ± 9.18 aA	20.07 ± 5.68 aA	30.33 ± 5.79 aA	0.00 ± 0.00 bA	0.00 ± 0.00 bA

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly different within rows, analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test) except days 2, 5, 15, and 35 for which ANOVA (Tukey's HSD post-hoc test) was used. Values not sharing an uppercase letter are significantly different within columns, analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test) except 5-300 and 10-300 for which ANOVA (Tukey's HSD post-hoc test) was used.

Table 4.5. Average putrescine concentrations (mg/100 g) of abalone during refrigerated storage.

Day	Control	5-100	10-100	5-300	10-300
2	1.13 ± 1.46 aC	1.19 ± 0.73 aC	0.00 ± 0.00 aD	0.00 ± 0.00 aA	0.00 ± 0.00 aA
5	0.00 ± 0.00 bBC	0.00 ± 0.00 bC	0.98 ± 0.20 aCD	0.00 ± 0.00 bA	0.00 ± 0.00 bA
10	16.64 ± 9.80 aBC	17.54 ± 0.93 aBC	13.66 ± 7.88 abCD	0.00 ± 0.00 bA	0.00 ± 0.00 bA
15	41.50 ± 8.47 abAB	53.28 ± 3.73 aABC	41.38 ± 6.75 abACD	0.00 ± 0.00 bA	0.00 ± 0.00 bA
21	25.12 ± 15.76 bABC	30.77 ± 8.09 bABC	99.51 ± 15.89 aABC	0.00 ± 0.00 bA	0.00 ± 0.00 bA
25	78.05 ± 17.53 bA	127.19 ± 11.24 aA	101.14 ± 11.37 abABCD	0.00 ± 0.00 cA	0.00 ± 0.00 cA
31	129.56 ± 20.02 abA	119.10 ± 18.59 abcA	144.06 ± 6.51 aAB	0.00 ± 0.00 bcA	0.00 ± 0.00 cA
35	113.49 ± 4.18 abA	98.49 ± 25.31 abAB	142.20 ± 22.80 aB	0.00 ± 0.00 bA	0.00 ± 0.00 bA

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly different within rows, analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test) except for days 21 and 25, for which ANOVA (Tukey's HSD post-hoc test) was used. Values not sharing an uppercase letter are significantly different within columns, analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test) except for 5-300 and 10-300 for which ANOVA (Tukey's HSD post-hoc test) was used.

4.3.5. Correlations

Aerobic plate counts had strong, significant ($p<0.01$) positive correlations with all four biochemical values. A positive correlation was expected since TVBN and biogenic amines are produced by bacterial action. Pressure had a strong, significant ($p<0.01$) negative correlation with aerobic plate counts, TVBN, and biogenic amine values, and a weak, significant ($p<0.01$) negative correlation with K-value and biochemical values. The relationship between pressure and shelf-life was expected since pressure at the 300 MPa level has been shown throughout the study to decrease aerobic plate counts and biochemical values significantly.

Table 4.6 Pearson correlation matrix. Treatments were analyzed by Pearson's Correlation with a Bonferroni Correction ($p<0.01$).

	Pressure	APC	TVBN	K-Value	Cadaverine
APC	-0.537				
TVBN	-0.591	0.746			
K-Value	-0.274	0.797	0.599		
Cadaverine	-0.601	0.707	0.863	0.533	
Putrescine	-0.664	0.696	0.915	0.540	0.920

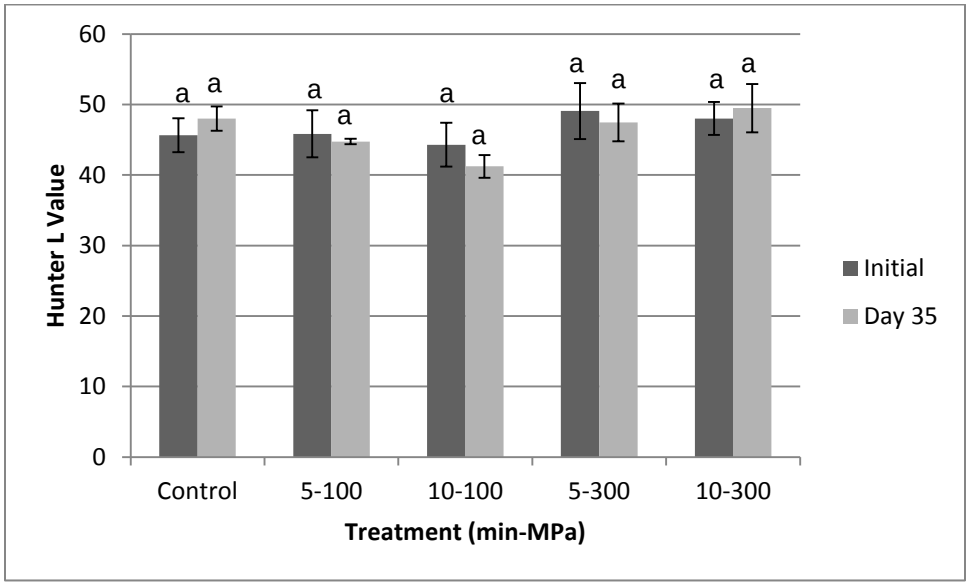
4.3.6. Color

The lightness (L) and whiteness (W) of the abalone did not change significantly ($p>0.05$) over the course of the 35 day shelf-life study for any treatment (Figures 4.6 and 4.9). The redness (a) and yellowness (b) of all treatments except the 10-100 similarly did not change over time, though both values decreased over time for the 10-100 treatment (Figures 4.7 and 4.8). Similarly, in a 60 day shelf-life evaluation of HPP abalone, L, a, and b values did not change in the first 45 days for abalones processed at 500-550 MPa, but the unprocessed abalones did decrease in all three color parameters over time

(Briones-Labarca and others 2012). The lightness and whiteness values at both time points ranged between 40 and 50 on the L and W scales, compared to 50 and 60 in chapter 2. The decrease is likely due to the accumulation of pigment in the foot of older abalones causing darker coloration compared to the younger abalones used in the previous chapters (2-3 years instead of 7-8 years).

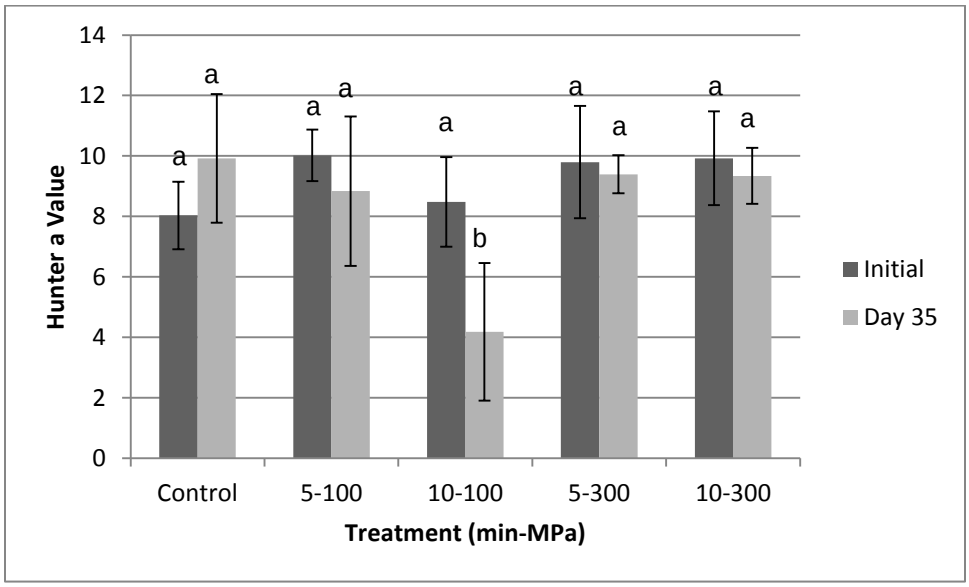
The redness values ranged from 8-10 on the a scale, but dropped to 4 for the Day 35 10-100 treatment. Similarly, the yellowness values ranged from 15-18 on the b scale, but dropped to 11 for the Day 35 10-100 treatment. The 10-100 treatment was not significantly different from the 5-100 or control treatments for biochemical or microbiological evaluations, so the reason for the significant drop in both a and b values for that treatment only is unknown, especially since initial values were very similar across the treatments and a and b values did not decrease with time in a similar study (Briones-Labarca and others 2010). Except for the 10-100 treatment, color was unaffected over 35 days suggesting that visual color degradation would not be a concern for a shelf-life of that length for either control or 300 MPa processed abalones. Sensory evaluation using a trained panel would be able to corroborate these findings since instrumental evaluations for color may not be sensitive to all consumer perceptions, such as gloss from polysaccharide production by bacteria over time.

Figure 4.6. Hunter L comparison of abalone initially and after 35 days.



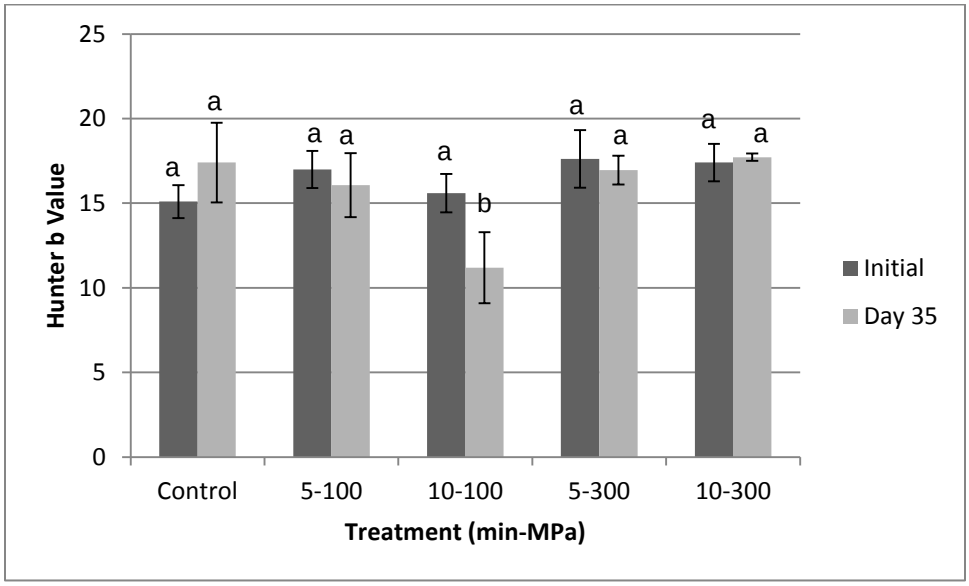
Each value is the mean ± standard deviation (n=12 for initial, n=3 for Day 35). Initial and final values not sharing a lowercase letter are significantly ($p<0.05$) different within a treatment, analyzed by ANOVA (Tukey's HSD post-hoc test).

Figure 4.7. Hunter a comparison of abalone initially and after 35 days.



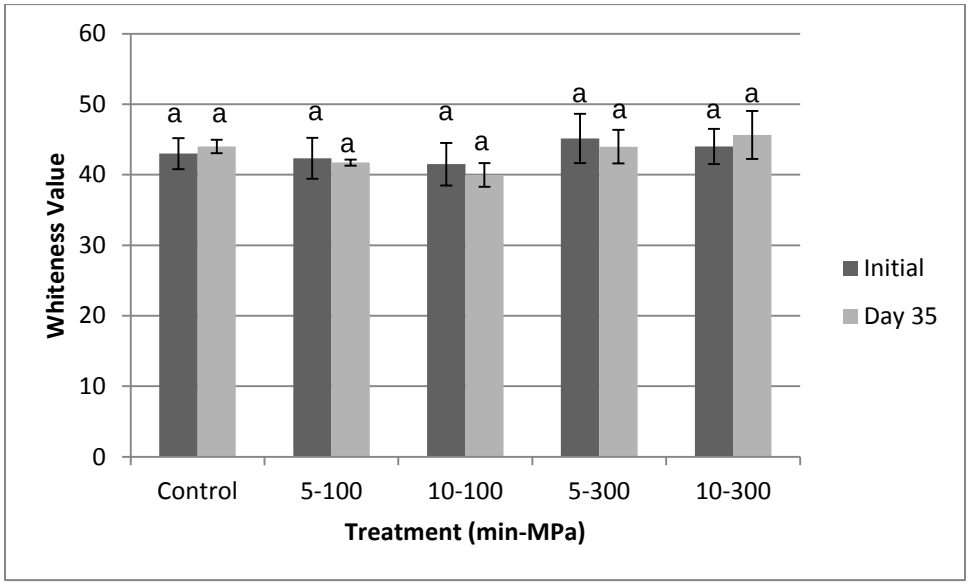
Each value is the mean ± standard deviation (n=12 for initial, n=3 for Day 35). Initial and final values not sharing a lowercase letter are significantly ($p<0.05$) different within a treatment, analyzed by ANOVA (Tukey's HSD post-hoc test).

Figure 4.8. Hunter b comparison of abalone initially and after 35 days.



Each value is the mean ± standard deviation (n=12 for initial, n=3 for Day 35). Initial and final values not sharing a lowercase letter are significantly ($p<0.05$) different within a treatment, analyzed by ANOVA (Tukey's HSD post-hoc test).

Figure 4.9. Whiteness comparison of abalone initially and after 35 days.



Each value is the mean ± standard deviation (n=12 for initial, n=3 for Day 35). Initial and final values not sharing a lowercase letter are significantly ($p<0.05$) different within a treatment, analyzed by ANOVA (Tukey's HSD post-hoc test).

4.3.7. Texture

Abalone firmness, chewiness, springiness, and resilience did not change after 35 days of refrigerated storage suggesting that texture was largely unaffected by time despite the microbial and biochemical changes that took place (Tables 4.6 and 4.7). Abalone cohesiveness was the only textural parameter to decrease with time, changing from an average of 608 to 528. There were no significant ($p>0.05$) differences among treatments within time points for any parameter, suggesting that the processing pressures and times used in this study did not affect the texture of abalone compared to an unprocessed control. These results are comparable to those presented in chapters 2 and 3 for raw, post-rigor processed abalone which did not differ significantly from unprocessed controls.

In contrast, in another HPP abalone study, texture was evaluated over 30 days and firmness decreased by 18 times between day 0 and day 30 for the control and by two times between day 0 and day 30 for the treatment processed at 500 MPa for 8 min (Briones-Labarca and others 2012). Significant decreases were also reported for chewiness, but there were no significant differences in springiness and cohesiveness after 30 days (Briones-Labarca and others 2012) which agreed with the findings in the present study when processed at less than 500 MPa. The drastic change in texture over time reported in the abalones processed at 500 MPa suggests increased proteolytic activity, though the TVBN values were similar between that study (22.37 mg N/100 g) and the present study described in this chapter (23.77 mg N/100 g) at Day 35. It may be that

pressures as high as 500 MPa cause structural changes in the myofibrillar proteins or connective tissues not detected by initial texture analysis that make those fibers more susceptible to enzymatic degradation and thus textural changes over time.

Each TPA parameter was comparable to values reported in chapter 2, except cohesiveness which was not evaluated in that study. Firmness values ranged from 3.608 N (5-100) to 7.852 N (10-300) initially, and 2.350 N (10-100) to 7.120 N (10-300) after 35 days. Chewiness values ranged from 1.593 (5-100) to 3.697 (10-300) initially, and 1.183 (10-100) to 4.064 (10-300) after 35 days. Springiness, cohesiveness, and resilience values were all under 1 initially and after 35 days. Overall texture of abalone, whether processed or not, did not change after the 35 day shelf-life suggesting, along with color data, that a 35 day shelf-life is suitable from a physical quality perspective. Sensory evaluation with a trained panel would be necessary to corroborate these findings since human sensitivities may detect changes that the TPA probe did not.

Table 4.7. Initial and Day 35 firmness (N) and chewiness TPA values.

	Firmness (N)		Chewiness	
	Initial	Day 35	Initial	Day 35
Control	3.914 ± 2.788 aA	3.478 ± 0.376 aA	2.223 ± 1.459 aA	1.333 ± 0.172 aA
5-100	3.608 ± 3.117 aA	3.357 ± 2.069 aA	1.593 ± 1.093 aA	1.332 ± 0.473 aA
10-100	6.302 ± 6.775 aA	2.350 ± 1.143 aA	2.397 ± 2.086 aA	1.183 ± 0.214 aA
5-300	4.538 ± 2.794 aA	5.573 ± 0.870 aA	2.208 ± 1.204 aA	2.904 ± 1.245 aA
10-300	7.852 ± 6.659 aA	7.120 ± 3.120 aA	3.697 ± 2.584 aA	4.064 ± 1.477 aA

Each value is the mean ± standard deviation (n=12 for initial, n=3 for 35 day). Values not sharing a lowercase letter are significantly different within rows for grouped columns (Firmness, Chewiness), analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test). Values not sharing an uppercase letter are significantly different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test).

Table 4.8. Initial and Day 35 springiness, cohesiveness, and resilience TPA values.

	Springiness		Cohesiveness		Resilience	
	Initial	Day 35	Initial	Day 35	Initial	Day 35
Control	0.929 ± 0.137 aA	0.764 ± 0.065 aA	0.592 ± 0.125 bA	0.505 ± 0.075 aA	0.275 ± 0.027 aAB	0.236 ± 0.012 aA
5-100	0.843 ± 0.115 aA	0.855 ± 0.148 aA	0.599 ± 0.105 bA	0.541 ± 0.131 aA	0.260 ± 0.062 aB	0.208 ± 0.021 aA
10-100	0.864 ± 0.147 aA	0.919 ± 0.081 aA	0.592 ± 0.142 bA	0.501 ± 0.169 aA	0.255 ± 0.047 aB	0.211 ± 0.028 aA
5-300	0.805 ± 0.115 aA	0.833 ± 0.089 aA	0.633 ± 0.065 aA	0.557 ± 0.086 aA	0.271 ± 0.038 aB	0.303 ± 0.027 aA
10-300	0.876 ± 0.093 aA	0.822 ± 0.070 aA	0.626 ± 0.087 bA	0.536 ± 0.064 aA	0.343 ± 0.104 aA	0.312 ± 0.002 aA

Each value is the mean ± standard deviation (n=12 for initial, n=3 for Day 35). Values not sharing a lowercase letter are significantly different within rows for grouped columns (Springiness, Cohesiveness, Resilience), analyzed by ANOVA (Tukey's HSD post-hoc test) except for resilience which was analyzed by Kruskall-Wallis (Mann-Whitney post-hoc test). Values not sharing an uppercase letter are significantly different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test).

4.4. Conclusions

The results of this study demonstrate that based on microbiological, biochemical, and physical evaluations of HPP abalone, a 25 day shelf-life can be achieved using 300 MPa for 10 min. K-value was the limiting factor of the biochemical tests, with values exceeding 50% after Day 25 for the 10-300 treatment. All of the other evaluations resulted in a 35 day shelf-life based on current industry recommendations for safe and good quality seafood products. These results are conditional upon sensory evaluation by a trained panel, since sensory rejection is often species and storage specific and overarching industry recommendations are intended as guidelines only.

Further, shelf-life evaluations of abalone, whether HPP or unprocessed, are limited so thorough comparisons with other conditions and species makes it difficult to make a conclusive shelf-life recommendation. Extension of abalone shelf-life beyond 1-2 weeks is useful for either home or industry use since currently abalone are sold live in-shell or preserved by freezing, drying, or canning. HPP appears to be an excellent mechanism to extend the shelf-life of raw, refrigerated abalone meat for up to 25 days.

CHAPTER 5

EFFECT OF PAPAIN ON THE PHYSICOCHEMICAL QUALITIES OF POST-RIGOR HIGH PRESSURE PROCESSED FARM-RAISED ABALONE (*Haliotis rufescens*)

5.1. Objectives

Papain is a common food-grade meat tenderizer that has been minimally explored for application on molluscan shellfish. It has been evaluated for canned whelk and abalone meat, however, it was not found to be an effective tenderizer at the specific concentration and activity used (Sanchez-Brambila and others 2002a, Sanchez-Brambila and others 2002b). Longer vacuum tumbling times, 20 min instead of 10 min, did produce more tender abalone meat at ambient temperature, though the lower values were still not significantly different from the untreated control (Sanchez-Brambila and others 2002b). The effect of vacuum tumbling time on tenderizing abalone despite a lack of significant difference from the control suggests some papain activity at ambient temperature despite its optimum activation temperature being 65ºC (Sanchez-Brambila and others 2002b).

High pressure processing (HPP) at 500 or 550 MPa has not been found to affect abalone texture, likely due to the high concentration of collagen generating greater structural integrity than in meat with less collagen (Briones-Labarca and others 2012). The high concentration of collagen in abalone meat may contribute to its resistance to papain tenderization, however it is known that high pressure processing (HPP) is capable of affecting collagen structure, if not quantity, in beef (Ichinoseki and others 2006). The

combination of HPP followed by papain treatment has not been previously evaluated, and if HPP affects collagen structure as predicted then the papain treatment may be more effective due to greater exposure of the collagen fibers to the proteolytic activity of papain. Additionally, increasing the papain solution temperature prior to tumbling may improve papain tenderization effectiveness. The objectives of this study were to evaluate 1) the effects of HPP at 300 MPa for 10 min, and 2) the effects of the same HPP parameters followed by treatment with two different high-activity papain concentrations (0.5% and 1.0% w/v at 35ºC) on physicochemical qualities of abalone meat.

5.2. Materials and Methods

The study had a 4x1 factorial design, assessing an unprocessed control (no HPP, no papain), an HPP control (no papain), and two HPP + papain treatments (0.5% and 1.0% w/v). All treatments, except the unprocessed control, were processed at 300 MPa for 10 min. Whole abalone meats were used for moisture, protein, and collagen evaluations. The foot muscle was used for color, texture, water holding capacity, protein gel electrophoresis, and scanning electron microscopy since it had been previously determined to be most affected by HPP compared to the adductor muscle. There were three processing replicates for each of the four treatments, with five abalones per replicate for a total of 60 abalones. Reagents were analytical grade and were purchased from Fisher Scientific (Waltham, MA) unless otherwise noted. Samples for collagen analyses were frozen after processing. Texture and color analyses and scanning electron microscopy (SEM) preparations were conducted the day after processing. SDS-PAGE

samples were frozen the day after processing for 30 days. Moisture content, water holding capacity, and collagen analyses were conducted three days after processing. Values are presented on a wet-weight basis (wwb).

5.2.1. Processing

Live farm-raised abalones (*Haliotis rufescens*) (n=60) (The Abalone Farm, Cayucas, CA) were divided according to the experimental design. Mean weight of the live, in-shell abalones was 315.6 g ± 97.4 and the mean shell length was 125.0 mm ± 14.3. Abalones had not been fed for a minimum of 4 days prior to shucking. Abalones were shucked, eviscerated, and scrubbed to remove epipodium pigment. The mean shucked, eviscerated meat weight was 88.9 g ± 9.1. Shucked meats were loosely packed 15 per bag (Winpak, Films Inc., Senoia, GA) and stored at 2°C for 24 h to allow mucus secretions to cease. Following the holding period, all abalones, including the controls, were rinsed in cool water and packed 4 per bag at 99% vacuum (Model UV550, Koch Industries, Wichita, KS). Bags were stored on ice during transport to the Natick Soldier Research, Development, and Engineering Center (Natick, MA). Control abalones were also kept on ice to mimic storage conditions of processed abalones.

A 1 L HPP unit (Engineering Pressure Systems Inc., Haverhill, MA) was used to process the bagged abalones at 300 MPa for 10 min. The temperature of the vessel during pressurization ranged from 20°C-26°C. Hydraulic fluid (20:1 water:Hydrolubric 120-B (Houghton International Inc., Norristown, PA)) was used to achieve hydrostatic pressure. The come-up time was 5 min and depressurization was immediate. Following HPP, all treatments, including the unprocessed control, were rinsed in cool water and re-

packed 5 per bag at 50% vacuum and stored at 2°C. Papain solutions were made immediately before application using 35°C water. Solution volumes were calculated based on a 4:1 ratio of volume:abalone weight, and consisted of 0.5% or 1.0% (w/v) papain (Acros Organics, Fair Lawn, NJ) in water with an activity of at least 46,000 units/mg, where one unit will hydrolyze 1.0 µmol of N-α-benzoyl-L-arginine ethyl ester per min at pH 6.2 at 25 °C (Sigma 2014). Treatment replicates were vacuum tumbled (Koch Industries, Wichita, KS) in the 35ºC papain solutions at -80 kPa for 20 min at 23 rpm. The solution temperature following tumbling was consistently 26ºC. Vacuum tumbled abalones were thoroughly rinsed in cool water, packed at 50% vacuum, and stored at 2°C for 15-20 h until commencement of analyses.

5.2.2. Moisture, pH, Protein, and Water Holding Capacity

Moisture content was determined gravimetrically by drying duplicate 3 g chopped samples 7 hrs in a 105°C oven (Jade Range, Brea, CA). The pH was taken from three samples per treatment (1:5 w/v in deionized water) and averaged. Protein content was determined in duplicate with a combustion nitrogen analyzer (Rapid N III, Elementar Americas Inc, Mount Laurel, NJ). Aspartic acid (Sigma-Aldrich, St. Louis, MO) was used as the nitrogen standard, and a conversion factor of 6.25 was used to determine crude protein content. Water holding capacity (WHC) was evaluated in duplicate. Abalone foot plugs were weighed, wrapped in 4 pieces of pre-weighed Whatman #1 filter paper, placed in 50 mL falcon tubes, and centrifuged at 1000 x *g* for 15 min in a bench top centrifuge (model 5430, Eppendorf, Hamburg, Germany). Following centrifugation,

filter paper was re-weighed and the difference was recorded. WHC was calculated as the percent of water retained by the meat with respect to water present in meat prior to centrifugation.

5.2.3. Collagen Content

Heat soluble and heat insoluble collagen contents of chopped whole abalone meat were evaluated following the methods of Hill (1966) and Ichinoseki and others (2006). Triplicate 4 g samples were added to 12 mL DI water in centrifuge tubes. Tubes were vortexed and placed in a water bath maintained at 77°C for 63 min (60 min + 3 min to allow meat to reach 77°C in center), swirling once halfway through. After heating, tubes were centrifuged (model J2-21, Beckman Coulter, Brea, CA) at 10,000 x *g* for 20 min using a chilled rotor. The supernatants were quantified and reserved at 4°C. To the pellet, ~8 mL DI water was added then vortexed and centrifuged again. The final supernatant was added to the first extraction and brought to 20 mL with DI water as the heat soluble fraction. The pellet was reserved at 4ºC as the heat insoluble fraction.

Hydrolysis of the heat soluble collagen fractions followed the method of Espe and others (2004). To 1 mL of each heat soluble collagen fraction, 1 mL 60% H_2SO_4 was added to complete acid hydrolysis over 12 h at 80ºC. Hydrolyzed samples were neutralized with 5 N NaOH to pH 6-7 then brought to volume in 50 mL quantitative flasks with DI water. Total collagen was evaluated following the AOAC (1995) method for hydroxyproline in meat. Meat from two abalones per treatment was chopped and 4 g of each were added to 30 mL 7 N H_2SO_4. Gelatin (Knox, Deerfield, IL) was used as an analytical control and 0.5 g in duplicate was added to 30 mL 7 N H_2SO_4 for hydrolysis.

Acidified samples were placed in an 80°C oven (Fisher Isotemp, Barrington, IL) for 16 h, cooled, and quantitatively brought to 500 mL with DI water. Solutions were filtered through Whatman #1 filter paper and stored at 4°C.

The hydroxyproline (Sigma-Aldrich, St. Louis, MO) standard curve was made fresh daily. The chloramine-T (Sigma-Aldrich, St. Louis, MO) oxidant solution, 4-dimethylaminobenzaldehyde (Sigma-Aldrich, St. Louis, MO) color reagent, and acetate-citrate buffer were made as described in the AOAC method. To each 0.25 mL aliquot of the final collagen dilution, 1.75 mL DI water was added to bring final test tube volume to 2 mL. Standard curve, control, and HPP control solutions were not diluted and 2 mL was used directly. To each test tube 1 mL oxidant solution was added, vortexed, and allowed to stand at room temperature for 20 min. To the oxidized sample, 1 mL color reagent was added, then the sample was vortexed, and immediately immersed in a water bath maintained at 60°C for exactly 15 min, cooled, and absorbance was read in a spectrophotometer (DU530, Beckman Coulter, Brea, CA) at 558 nm. Hydroxyproline concentration (g/100 g) was determined by comparing sample absorbance to the hydroxyproline standard curve. The collagen content (g/100 g) was calculated using a conversion factor of 9.8 since abalone collagen is reported to contain 10.2% hydroxyproline (Kimura and Kubota 1968).

5.2.4. Colorimetric and Texture Analyses

Treatments were analyzed in random order. The whole abalones were sliced horizontally below the base of the adductor to separate the foot from the adductor muscle. Square plugs measuring 20x20x10 mm by a digital caliper were cut from the center of the

ventral side of the foot. Plugs were stored in 7.5 x 12.5 cm WhirlPak (Nasco, Fort Atkinson, WI) bags at 4°C until analyses.

5.2.4.1. Color

Colorimetric analyses (n=5 per treatment) were performed using a colorimeter (LabScan XE, Hunter Labs, Reston, VA). The Hunter L, a, b values were recorded by the colorimeter software (Universal, version 4.10, 2001, Hunter Labs, Reston, VA). The colorimeter was standardized using white and black tiles. Each plug was evaluated in triplicate on a watch glass using the 10º/D65 setting, a port area of 17 mm, and an area view of 25.4. Triplicate readings were averaged for each sample.

5.2.4.2. Texture

Following color analysis, individual plugs (n=5) were placed on the calibrated texture analyzer platform (TA-XTi2, Texture Technologies Inc., Scarsdale, NY) non-cut surface side up and positioned so the Warner-Bratzler v-notch blade cut across the muscle fibers. The texture analyzer was configured to a 30 mm distance, a 1.5 mm/s test speed, and a trigger force of 0.3922 N. Units of force were given in Newtons (N), recorded by the texture analyzer software (Exponent 32, version 5,0,6,0 2010, Texture Technologies Inc., Scarsdale, NY). Samples were kept at 4°C prior to evaluations.

5.2.5. SDS-PAGE

Following texture analysis, the sheared pieces of meat were vacuum packaged and frozen at -80ºC until sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). To solubilize the samples, they were first thawed at 4ºC then cut into small

pieces weighing 0.0017 g (control), 0.0016 g (HPP control), 0.0016 g (0.5% papain), and 0.0009 g (1.0% papain). A second set of the papain treatments was prepared using a four times higher meat mass at 0.0067 g (0.5% papain), and 0.0067 g (1.0% papain). Each piece was added to a microcentrifuge tube with 1 mL SDS-urea solution (2% SDS (Sigma-Aldrich, St. Louis, MO), 8 M urea (Sigma-Aldrich, St. Louis, MO), 2% 2-mercaptoethanol (J.T. Baker, Phillipsburg, NJ), 50 mM tris-HCl (Sigma-Aldrich, St. Louis, MO)), vortexed, and immersed in boiling water until solubilized. All papain treatments were solubilized within 10 min while it took 90 min to dissolve the control and HPP control samples. The higher mass (0.0067 g and 0.0067 g) papain treatments were removed from the boiling water at 10 min. The lower mass (0.0009 g and 0.0016 g) papain treatments were boiled for 90 min to compare directly to the control and HPP treatment. Solubilized samples were frozen at -15ºC until gel electrophoresis.

Gels were prepared using a 4% stacking solution and a 10% resolving solution (Laemmli 1970). The wells were loaded with 7.5 µL molecular weight reference ladder (10-200 kDa) and 15 µL of each solubilized sample. Gels were run at 180 V and 100 mA for 60 min in a Mini-PROTEAN 3 Cell (Bio-Rad, Hercules, CA), then carefully stained in 0.125% Coomassie blue in 50% methanol, 10% acetic acid, and 40% DI water for 4 h. Gels were destained in 45% methanol, 10% acetic acid, and 45% DI water over 15 h, rinsed several times with deionized (DI) water, and stored at ambient temperature in DI water. Gels were photographed using a Luminscent Image Analyzer (FujiFilm, Valhalla, NY) equipped with LAS-4000 (FujiFilm, Valhalla, NY) and Multigauge (FujiFilm, Valhalla, NY) software for digital image capture.

5.2.6. Scanning Electron Microscopy

Abalone samples were prepared for scanning electron microscopy (SEM) by slicing 5x5x5 mm cubes (n=5 per replicate) from the center of the ventral side of the foot muscle using a razor blade. Stock 0.2 M phosphate buffer was made by combining 0.22 g $NaH_2PO_4 \cdot H_2O$, 1.19 g Na_2HPO_4, and 8 g sucrose in 50 mL deionized water to a final pH of 7.3. Working buffer was made by making a 1:1 ratio of stock phosphate buffer:deionized water to a final concentration of 0.1 M, with the addition of 1 drop 1% $CaCl_2$ per 10 mL buffer. Specimens were placed one cube per vial and fixed in approximately 3 mL 2.5% glutaraldehyde (Electron Microscopy Services, Hatfield, PA) solution for 4 days at 4°C.

The initial fixation was followed by a replacement of fixative with working buffer and a consolidation of specimens to 2 or 3 per vial. Specimen vials were iced throughout rinsing and final fixation. The buffered specimens were rinsed twice in a pre-warmed microwave oven using two 7/20/7 cycles (7 s on high, a 20 s rest, and a final 7 s on high) with buffer replacement between the two cycles. The rinsed specimens were then fixed with 1% OsO_4 (Electron Microscopy Services, Hatfield, PA) for one 7/20/7 cycle and rinsed briefly with deionized water at ambient temperature. Fixed specimens were dehydrated in serially increasing concentrations of ethanol (50%, 70%, 95%) for two 7/20/7 cycles at each level, with ethanol replacement between each cycle. Following the 95% dehydration, samples were further dehydrated in 100% ethanol three times for 7 min each time at ambient temperature. The specimens were stored in 100% ethanol at 4°C until freeze drying.

Freeze drying was accomplished by t-butyl alcohol sublimation. Ethanol was replaced by 30ºC t-butyl alcohol for a total of two rinses. The t-butyl alcohol soaked specimens were chilled to below the 25ºC freezing point of t-butyl alcohol to quickly freeze the samples. The frozen samples were placed in the bell jar of a vacuum evaporator for several hours until sublimation was achieved. The dried samples were transferred to stubs affixed with carbon-coated tape and silver adhesive (503, Electron Microscopy Sciences, Hatfield, PA). The samples were sputter coated (Cressington 108 Auto, Redding, CA) at 40 mA and 0.08 mbar for 90 s to generate a 35 nm layer of gold-palladium on the surface. Samples were stored in a dessicator until imaging. The scanning electron microscope (AMRay 1820 Digital SEM, Bedford, MA) was degaussed initially and between samples. An accelerating potential of 10 kV and a spotsize of 10 were selected, and magnification up to 1,000 times (1000x) was used to visualize the ultrastructure of specimens.

5.2.7. Statistical Analyses

Data were analyzed using SYSTAT 12 (Systat Software, Chicago, IL) for one-way analysis of variance (ANOVA) for all one-level (treatment) analyses. Tukey's Honest Significant Difference (HSD) test was selected for post-hoc analyses except where Tukey's was too conservative to find differences identified by ANOVA, in which case Fisher's Least Significant Difference (LSD) test was used. The Shapiro-Wilk normality test and Levene equality of variances test were used to assess data prior to analyses. In cases where data did not satisfy either normality or homogeneity, they were

evaluated non-parametrically using Kruskal-Wallis. Mann-Whitney was selected for non-parametric post-hoc analyses. For all statistics, a significance level of $p<0.05$ was selected.

5.3. Results and Discussion

5.3.1. Moisture, pH, Protein, and Water Holding Capacity

There were no significant ($p>0.05$) differences in moisture, pH, or protein contents of the four treatments, indicating that neither HPP nor papain treatment affected those parameters (Table 5.1). Moisture content was ~75%, which was similar to values reported in previous chapters and in other studies, however it was expected that the HPP treatments would increase in moisture due to hydration of proteins from extracellular fluid (Olley and Thrower 1977, Briones-Labarca and others 2012). The consistent pH, ranging from 6.3-6.5, was unexpected since it has been reported for both abalone and oyster that HPP decreases pH due to protein denaturation and deamination (Lai and others 2010, Briones-Labarca and others 2012). However, higher pressures were used in those studies (500-550 MPa) than in this study, so it is possible the lower processing pressures in the current study (300 MPa) prevented significant changes in both pH and moisture. The protein content was ~25% which was much higher in this study than in previous chapters, but similar to reports of abalone harvested in California (Olley and Thrower 1977). The higher protein content of all treatments was likely due to the three times larger size and greater age of the abalones used in this study, as well as the fact that they had been part of a breeding study previous to their use in this study.

The water holding capacity (WHC) of the abalone meat was not significantly different between the control and the HPP control, averaging 90%, further supporting conclusions drawn in previous chapters that 300 MPa does not affect the texture of abalone (Table 5.1). There was a significant decrease in WHC in the papain treatments compared to the control, from 91.6 g/100 g to 84.0 g/100 g, though there was no effect of increasing papain concentration on WHC. The decrease in WHC was expected as the proteolytic activity of papain cleaved proteins and subsequently reduced their ability to retain water, which may affect perceptions of tenderness and juiciness in meat (Damodaran 2008).

Table 5.1. Moisture (g/100 g), pH, protein (g/100 g), and water holding capacity (WHC) (%) of abalone.

	Moisture	pH	Protein	WHC
Control	75.3 ± 0.6 a	6.33 ± 0.10 a	25.0 ± 2.1 a	91.6 ± 1.1 a
HPP Control	74.8 ± 0.4 a	6.56 ± 0.18 a	25.1 ± 1.9 a	89.5 ± 1.1 ab
0.5% Papain	74.9 ± 1.0 a	6.50 ± 0.09 a	25.3 ± 1.0 a	84.0 ± 2.6 b
1.0% Papain	74.8 ± 0.2 a	6.42 ± 0.04 a	25.7 ± 1.4 a	85.5 ± 0.7 b

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test) for moisture, pH, and protein, and Kruskall-Wallis (Mann-Whitney post-hoc test) for WHC.

5.3.2. Collagen Content

The total collagen content of each treatment was not significantly ($p>0.05$) different, at ~2 g per 100 g abalone meat, however there were very significant ($p<0.05$) differences among the treatments for heat soluble and insoluble collagen (Table 5.2). It was clear from all three types of collagen quantified that HPP did not affect the quantity or solubility of collagen. Heat soluble collagen was very low in relation to total collagen, at 0.161 g/100 g for the control and 0.073 g/100 g for the HPP control. Heat insoluble

collagen, however, represented the majority of collagen in control and HPP control at 1.817 g/100 g and 1.713 g/100 g, respectively. The high concentration of heat insoluble collagen in abalone meat is likely a strong contributor to the texture of abalone and the long boiling times required to tenderize the meat (Hatae and others 1996, Gao and others 2001).

Table 5.2. Collagen contents (g/100 g) of abalone.

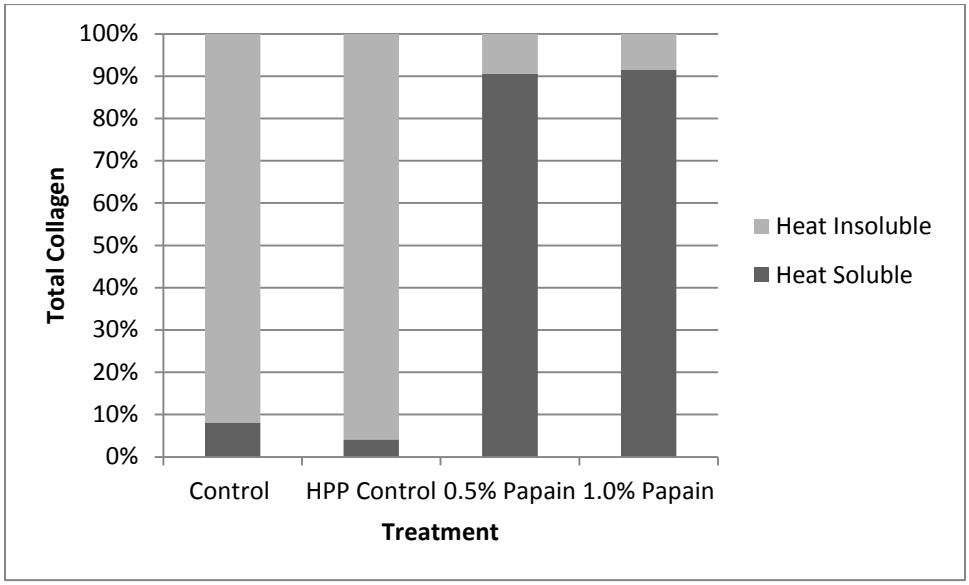
	Total	Heat Soluble	Heat Insoluble
Control	1.89 ± 0.24 a	0.16 ± 0.03 b	1.82 ± 0.09 a
HPP Control	2.11 ± 0.30 a	0.07 ± 0.02 b	1.71 ± 0.16 a
0.5% Papain	1.89 ± 0.37 a	2.46 ± 0.84 a	0.26 ± 0.09 b
1.0% Papain	1.76 ± 0.09 a	1.98 ± 0.43 a	0.18 ± 0.04 b

Each value is the mean ± standard deviation (n=3). Values not sharing a lowercase letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test).

There was a significant effect of the HPP + papain treatment on the solubility of collagen, with 90% of the total collagen becoming heat soluble compared to the control and HPP control which contained less than 10% heat soluble collagen (Figure 5.1). The observed changes in collagen ultrastructure in chapter 2, where collagen fibrils were evident after HPP, suggested that papain used after HPP may be more successful at tenderizing abalone meat than papain by itself due to the increased surface area of the collagen. The discrepancy in values from total collagen to the sum total of the heat soluble and heat insoluble collagen may be due to the number of pre-hydrolysis steps in the soluble collagen method. The total collagen method requires no pre-treatment and so is probably more accurate because there is less room for error. Finally, the homogenization of the abalone meat prior to subsampling may have contributed to slight differences between total and the soluble collagen method results.

Though the heat soluble and heat insoluble values do not equal the values for total collagen, the sharp difference between the controls and HPP + papain treatments supports the conclusion that based on these effects on collagen solubility, it would appear that the increased surface area of the HPP abalone collagen fibrils may have made it more susceptible to the proteolytic activity of papain, and thus changed its heat solubility.

Figure 5.1. Heat solubility of collagen.

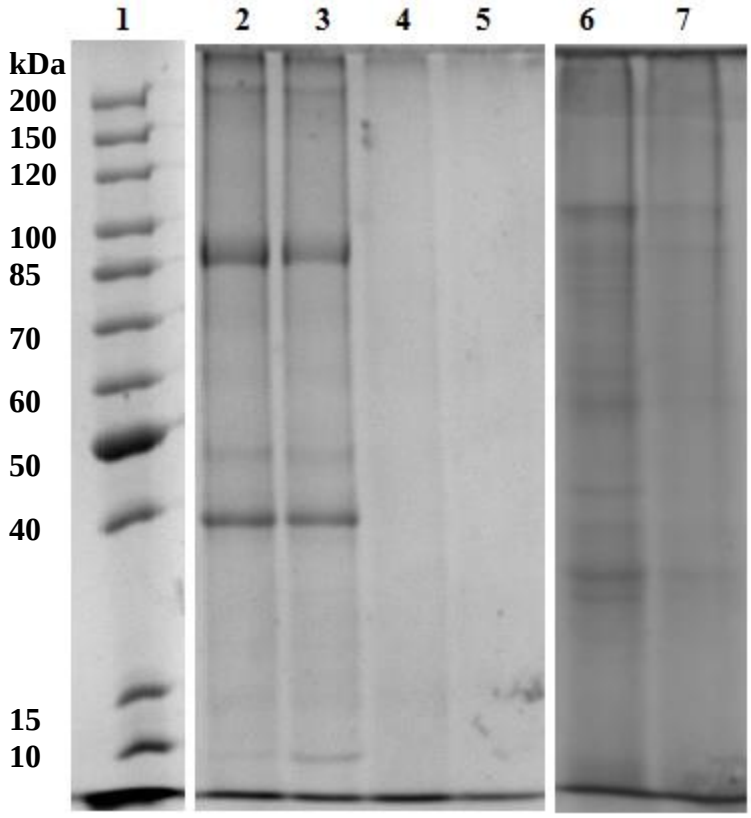


5.3.3. SDS-PAGE

SDS-PAGE confirmed the collagen solubility results by showing a distinct difference in protein profile between the two controls and the two HPP + papain treatments (Figure 5.2). It appears that there was no difference between the control (lane 2) and the HPP control (lane 3), with both having distinct lines at 40 kDa and 85 kDa, suggesting that HPP does not affect the molecular weight of abalone proteins. The 40 kDa protein band may be either troponin (37 kDa) or actin (42 kDa) (Strasburg and others 2008). The 85 kDa protein band may be collagen type 1 chain B (93 kDa) (Ha and

others 2012). By contrast, protein bands were completely absent in the HPP-papain treatments in lanes 4 and 5, which had a similar quantity of protein and were boiled for the same amount of time as the controls. The smaller peptides of the papain treatments, evidenced in lanes 6 and 7 which had four times the quantity of protein and were boiled only 10 min, eluted throughout the lanes in the range of 20-100 kDa without clear distinction. The lack of clear protein bands demonstrates extensive proteolysis of the abalone meat following the HPP + papain treatment. There did not appear to be notable differences as a result of papain concentration, so the 0.5% was as effective as the 1.0% at hydrolyzing muscle and decreasing the molecular weight of the meat proteins.

Figure 5.2. SDS-PAGE.



Lane 1 is the reference standard (10-200 KDa). Lane 2 is control (0.0017 g) boiled 90 min. Lane 3 is HPP control (0.0016 g) boiled 90 min. Lane 4 is 0.5% papain (0.0016 g) boiled 90 min. Lane 5 is 1.0% papain (0.0009 g) boiled 90 min. Lane 6 is 0.5% papain (0.0067 g) boiled 10 min. Lane 7 is 1.0% papain (0.0067 g).

5.3.4. Color

The control was significantly ($p<0.05$) less light and less yellow than the other treatments, supporting evidence in previous chapters of the bleaching effect of HPP (Table 5.3). There were no significant differences in color due to subsequent papain treatment. L values for the control averaged 27.80 compared to 36.34 for the HPP control and 38.5 for the papain treatments. The a values ranged from 4.55 (control) to 5.69 (HPP control) but were not significantly different among treatments, suggesting that

neither HPP nor papain had an effect on red pigments. The b values were 8.22 for the control, but proportionally increased with the L values to 12.48 (HPP control), 12.63 (0.5% papain), and 13.01 (1.0% papain). An increase in L and b values following HPP has been reported in many seafood products, including salmon, mahi mahi, and trout, though decreases have been reported for abalone (Yagiz and others 2007, Yagiz and others 2009, Briones-Labarba and others 2012). Static a values of HPP abalone compared to unprocessed abalone have been reported previously, though decreased a values have been reported for fish (Yagiz and others 2007, Yagiz and others 2009, Briones-Labarba and others 2012). The high carotenoid content of trout and salmon compared to abalone may account for the decrease in a values observed in those species since lipid oxidation can be accelerated by high pressure and carotenoids are sensitive to oxidative bleaching (Yagiz and others 2007).

The overall color of all abalones in this study was much darker than in previous chapters, which may be due to the age and diet of the abalones. In previous studies, the abalones were 2-3 years old and fed fresh kelp while in the current study the abalones were 7-8 years old and fed dried pellets (Neil Greenberg, personal communication). It has been reported for Blacklip abalone that those fed artificial diets had lighter pigmentation than those fed algae, however it is plausible that over time there is a greater accumulation of pigmentation than in young abalone irrespective of diet (Allen and others 2006).

Table 5.3. Hunter L, a, b values.

	L	a	b
Control	27.80 ± 1.07 b	4.55 ± 0.48 a	8.22 ± 0.79 b
HPP control	36.34 ± 0.29 a	5.69 ± 1.04 a	12.48 ± 0.92 a
0.5% papain	38.58 ± 1.43 a	5.28 ± 0.84 a	12.63 ± 0.99 a
1.0% papain	38.50 ± 2.63 a	5.14 ± 0.93 a	13.01 ± 0.80 a

Each value is the mean ± standard deviation (n=5). Values not sharing a lowercase letter are significantly ($p<0.05$) different within columns, analyzed by ANOVA (Tukey's HSD post-hoc test).

5.3.5. Texture

There was a significant ($p<0.05$) 10 N decrease in force required to shear the abalone from the papain treatments compared to the control and HPP control (Table 5.4). The control and HPP control were not significantly different, at 30.913 N (control) and 31.739 N (HPP control), supporting the texture results of previous chapters in which HPP at 300 MPa for 10 min did not affect post-rigor HPP abalone foot meat. The papain treatments following HPP had a tenderizing effect, at 20.549 N (0.5% papain) and 19.867 N (1.0% papain), though concentration did not have a significant effect. Canned whelk pre-treated with 0.25% papain experienced an increase in shear force, while canned abalone pre-treated with 0.25% or 0.50% papain had shear force values of 2.58 N and 2.52 N respectively, compared to 2.45 N for the untreated canned control (Sanchez-Brambila and others 2002a, Sanchez-Brambila and others 2002b).

The much lower shear force values for the canned abalone compared to the raw abalone in the present study evidence the effect of high temperature on gelatinization of collagen and coagulation of myofibrillar proteins that occur during canning (Sanchez-Brambila and others 2002b). The absence of papain tenderization of canned abalone may have been due to not reaching optimum activation temperature of papain (65ºC) during

initial vacuum tumbling, which took place at ambient temperature. By contrast, a significant tenderization effect of papain was observed in the present study by pre-heating the papain solution to 35ºC to increase papain activity. Additionally, the use of a 4:1 papain solution:abalone weight ratio increased surface interaction, and the use of a higher-activity papain (46,000 units/mg compared to 16,000 units/mg) improved the efficacy of papain to tenderize abalone meat. HPP and subsequent 0.5% papain treatment effectively reduced shear force by 10 N, tenderizing the meat compared to unprocessed and processed controls. Future studies focusing on sensory evaluation of HPP and papain treated abalone are warranted to determine acceptance of the more tender meat as well as potential changes in flavor profile.

Table 5.4. Warner-Bratzler shear force (N).

	Force (N)
Control	30.913 ± 2.907 a
HPP Control	31.739 ± 7.458 a
0.5% Papain	20.549 ± 3.846 b
1.0% Papain	19.867 ± 3.851 b

Each value is the mean ± standard deviation (n=5). Values not sharing a lowercase letter are significantly ($p<0.05$) different, analyzed by ANOVA (Fisher's LSD post-hoc test).

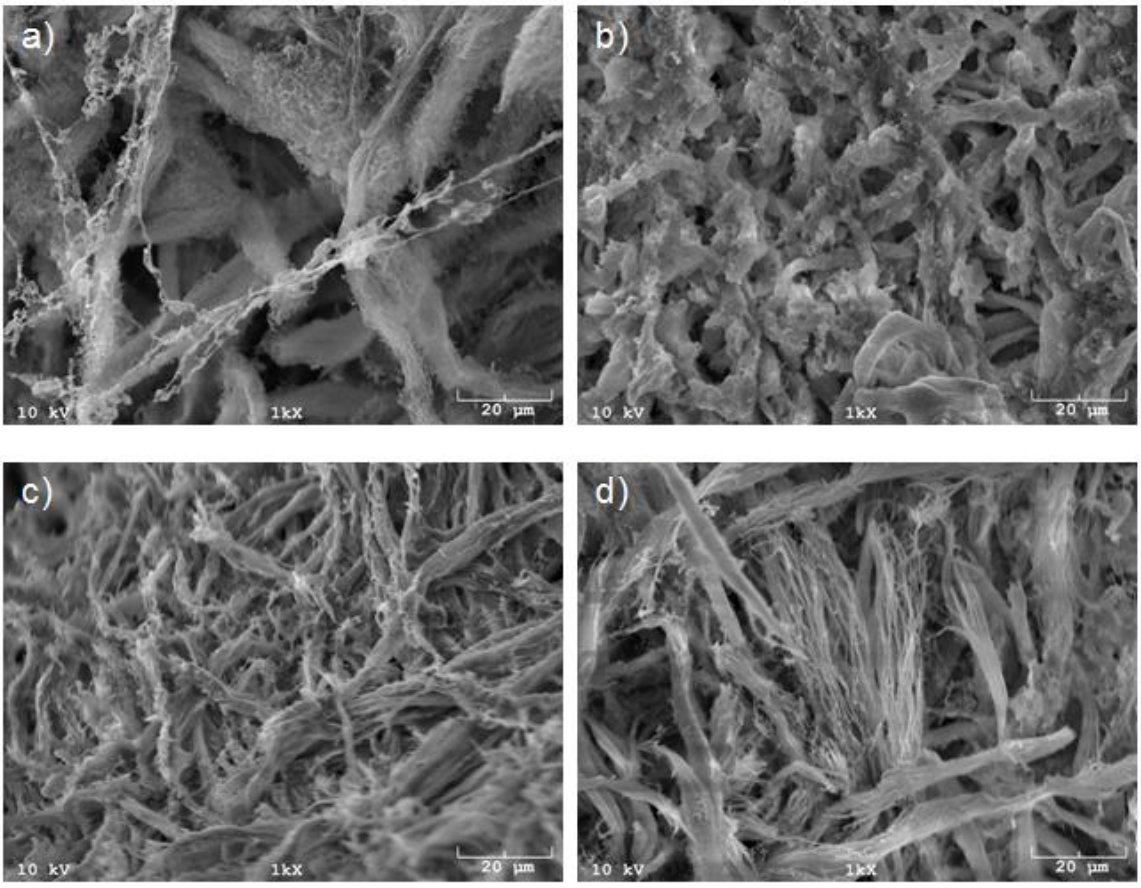
5.3.6. Scanning Electron Microscopy

Scanning electron microscopy (SEM) of the abalone collagen fibers allowed direct visual comparison of the treatment effect of HPP and HPP followed by papain treatment at two concentrations. The collagen fibers in the control were about twice as large as the collagen fibers in the HPP control (Figures 5.3a and 5.3b), suggesting a compaction of the fibers due to pressure, even though there were no significant effects in texture between the two treatments as measured by shear force values. Stretching of the

collagen matrix, as observed in HPP beef collagen (Ichinoseki and others 2006), was not observed despite following the same alkali cell maceration method to expose the honeycomb-shaped collagen matrix. Freezing the beef prior to fixation and alkali digestion may have increased efficacy of the solutions in exposing the collagen sheath matrix, however, SEM images of unfrozen fixed abalone (Hatae and others 1995) demonstrated collagen fibers that appear similar to those in the current study.

The effect of papain on HPP abalone, evidenced by the changes in collagen solubility, protein molecular weights, and texture, is further observed by the fraying of the collagen fibers into collagen fibrils (Figures 5.3c and 5.3d). Though significant differences between the two concentrations of papain (0.5% and 1.0%) were not found in the other analyses, it appears in the SEM images that the 1.0% caused more fraying with very distinct individual fibrils present. These images validate the physicochemical results already discussed, and indicate that the HPP + papain treatments worked to tenderize abalone meat. A follow-up study with a papain-only treatment (no HPP) would be necessary to validate the hypothesis that HPP + papain increase tenderness of abalone more than a papain-only treatment.

Figure 5.3. Scanning electron micrographs of abalone collagen. Unprocessed control (a), HPP control (b), HPP-0.5% papain (c), and HPP-1.0% papain (d) taken at 1000x. Scale bars represent 20 µm.



5.4. Conclusions

In conclusion, the combination of HPP at 300 MPa for 10 min and 0.5% papain, warmed to 35ºC, successfully tenderized abalone meat. Multiple physicochemical attributes were assessed and each led to the same conclusion, that high pressure alone did not produce differences in texture or functionality compared to the unprocessed control, however, vacuum tumbling the HPP abalone meat for 20 min at a 4:1 ratio of 0.5% papain solution:abalone weight produced more tender meat with greater heat solubility and collagen disruption. Evaluation of lower levels of papain, as well as a papain-only

treatment, would be useful to further conclusions about the ability of papain to tenderize abalone meat. Consumer acceptance tests of papain-treated HPP abalone meat is warranted to assess these changes in texture as well as any potential flavor changes to inform whether this process will be useful in adding value to the abalone industry.

CHAPTER 6

OVERALL CONCLUSIONS

These studies show that high pressure processing (HPP) is an effective non-thermal processing method that could provide competitive advantage to the abalone aquaculture industry. Abalone rigor status during HPP had a significant effect on abalone quality, with pre-rigor processed abalone exhibiting toughening compared to the unprocessed abalone as well as to the post-rigor processed abalone, irrespective of HPP time or pressure. The color of post-rigor processed abalone was also improved compared to the control, with increased L values. A post-shucking hold time of at least 26 h at 4ºC is consequently recommended prior to HPP of abalone to prevent toughening during processing. Additionally, disclosure of rigor status in seafood research, as is regularly done with meat research, is important so that proper comparisons and conclusions can be drawn given the importance of rigor status to physical qualities.

The effect of HPP on subsequently boiled meat has not been previously reported, and it was unknown whether HPP would cause a change in the cooked product. Toughening of HPP abalone was not observed in raw or cooked meats, though cooked HPP meat exhibited significantly higher L values than the cooked control, further confirming HPP as an excellent method to increase whiteness of abalone meat to improve consumer acceptance and market value. Further studies evaluating other cooking methods and consumer acceptance will be important to inform the abalone industry about the benefits of HPP with regard to subsequently cooked abalone meat.

Abalone destined for raw markets, either retail, restaurant, or wholesale, could benefit from HPP at 300 MPa for 10 min to improve shelf-life by 2-3 times. Assessing raw refrigerated HPP abalone over 35 days showed that the meat achieved a biochemical and microbiological shelf-life of 25 days. Texture and color were not affected by storage time, but sensory evaluation by a trained panel is recommended for future studies to corroborate laboratory-based shelf-life estimations. Such significant improvement in shelf-life, from 5-7 days to 25 days, could have substantial implications for an industry heavily dependent on international exports. Shucked HPP abalone could be exported without the added weight of the shell and viscera, or concern for mortality of live shipments, and reduce urgency of sales upon arrival. Domestically, the development of a raw refrigerated abalone market could have benefit for both consumers and restaurants.

An alternative to mechanical tenderization of abalone meat may be HPP followed by vacuum tumbling with a 35ºC 0.5% papain solution at a ratio of 4:1 papain solution:abalone weight. In addition to reducing shear force, the HPP and papain combination increased the heat solubility of abalone collagen from 10% to 90% which could have a significant impact on the texture of subsequently cooked abalone. The use of SDS-PAGE allowed the visualization of the extent of proteolysis that occurred due to papain activity, with numerous small molecular weight peptides taking the place of two significant protein bands at 40 kDa and 85 kDa. Scanning electron microscopy further validated physicochemical results by showing the unraveling of the collagen fibers. The increase in surface area of the collagen due to the exposure of individual fibrils in conjunction with the digestion of protein from the papain may account for the significant reduction in shear force as well as the change in collagen solubility.

In conclusion, the effects of HPP are positive for abalone meat by improving the color, shelf-life, and possible improvement in the susceptibility of abalone for proteolytic enzyme tenderization. HPP at the pressures and times evaluated did not affect texture of the meat, which could be important for a raw refrigerated abalone market. Rigor status of abalone meat is a critical factor, and processing post-rigor abalone will prevent textural changes during processing while still achieving color and shelf-life goals.

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